Systematic Revision of the Monotypic Family Orobdellidae (Hirudinida: Arhynchobdellida: Erpobdelliformes), with Molecular Phylogenetic Analyses of the Known Orobdellid Species

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This thesis is not issued for purposes of zoological nomenclature.

Abstract

A systematic revision of the monotypic family Orobdellidae is presented. The family is rediagnosed, and all of the known species of the genus *Orobdella* Oka, 1895, *viz*., *O*. *whitmani* Oka, 1895, *O*. *dolichopharynx* Nakano, 2011, *O*. *esulcata* Nakano, 2010, *O*. *ijimai* Oka, 1895, *O*. *kawakatsuorum* Richardson, 1975, *O*. *ketagalan* Nakano & Lai, 2012, *O*. *koikei* Nakano, 2012, *O*. *mononoke* Nakano, 2012, *O*. *octonaria* Oka, 1895, *O*. *shimadae* Nakano, 2011, and *O*. *tsushimensis* Nakano, 2010, are rediagnosed and redescribed on the basis of the examination of type specimens and additional materials. The phylogenetic relationships of these eleven species were also estimated using maximum-likelihood and Bayesian analyses of mitochondrial COI, and $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$ and 16S rRNA sequence data from 84 specimens of *Orobdella*. The genus *Orobdella* was divided into two main clades (A, B). Clade A consisted of two species distributed in Hokkaido, *O*. *kawakatsuorum* and *O*. *koikei*. Clade B comprised all of the remaining nine species that inhabited the area south of Hokkaido. Within Clade B, four main subclades with strong nodal supports were detected (B1–B4). Clade B1 contained five species, *O*. *esulcata* from Kyushu, Japan, *O*. *dolichopharynx*, *O*. *mononoke* and *O*. *shimadae* from the Ryukyu Islands, and *O*. *ketagalan* from Taiwan. Clade B2 consisted of two species, *O*. *ijimai* and *O*. *octonaria*, from Honshu, Japan. Clade B3 included only one species, *O*. *tsushimensis*, from Tsushima Island, Japan, and Gageodo Island, Korea. Clade B4 was also composed of one species, *O*. *whitmani*, from Honshu and Shikoku, Japan. Clade B4 was subdivided into three clades (a–c): Clade B4a contained *O*. *whitmani* from the Chugoku Region and Shikoku; Clade B4b consisted of *O*. *whitmani* from the central part of Honshu and included a specimen from the type locality; Clade B4c consisted of leeches from the Kinki Region. The relationships among Clades B1–B4 were not resolved statistically. On the basis of the present phylogenies, the annulation, the morphology of the gastroporal duct, and the degeneration of epididymides are likely to have evolved in parallel within the *Orobdella*. Intra- and interspecific K2P distances of COI sequences were also calculated. The results of the morphological examination, phylogenies, and the K2P distances obtained suggest that *O*. *whitmani* might contain two cryptic species (Clades B4a, and B4c), and that *O*. *dolichopharynx* might also include a cryptic species or subspecies (an individual from Tokunoshima Island in the Ryukyu Islands). To resolve the systematic position of these cryptic clades within *Orobdella*, additional

specimens are needed to sufficiently determine their morphological characteristics. Morphological comparisons and an identification key of the eleven known *Orobdella* species are provided. Six quadrannulate species are distinguished from each other by the following combination of characters: annulation of IV; annulation of XXIV; number of annuli between male and female gonopores; morphology of gastroporal duct; length of epididymides; with or without paired sperm duct bulbs; morphology of ejaculatory ducts in position anterior to ovisacs; and morphology of atrial cornua. Four sexannulate *Orobdella* leeches differ from each other in the following characters: annulation of III; annulation of IV; annulation of VI; annulation of VII; annulation of VIII; annulation of XXVI; number of annuli between gonopores; length of pharynx; morphology of gastroporal duct; length of epididymides; morphology of epididymides in position anterior to ovisacs; with or without pre-atrial loop; and morphology of atrial cornua.

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Introduction

Leeches (subclass Hirudinida) are hermaphrodite annelids that are closely related to oligochaetous worms. According to the classification by Sawyer (1986) with taking into account revisions in recent systematic studies (Borda and Siddall 2004; Phillips and Siddall 2009), Hirudinida is subdivided into two orders, *viz*., Rhynchobdellida with a proboscis in the foregut, and Arhynchobdellida without a proboscis. Arhynchobdellid leeches are classified into two suborders, *viz*., Hirudiniformes including dentate taxa with a euthylaematous pharynx straightly reaching to a crop, and Erpobdelliformes, with a strepsilaematous pharynx reaching to a crop with a rotation of 60 degrees to the right, taxa. Approximately 700 hirudinid species have been identified worldwide, and about 70 species have been recorded from Japan (Sket & Trontelj 2008).

The genus *Orobdella* Oka, 1895 is a Far Eastern genus of terrestrial macrophagous leeches, which belongs to Arhynchobdellida (Sawyer 1986). *Orobdella* species are large leeches that feed on earthworms, and mature *Orobdella* species are usually around 10 cm in length. This genus was established by Oka (1895), who also described three new species, *O*. *whitmani* Oka, 1895, *O*. *ijimai* Oka, 1895, and *O*. *octonaria* Oka, 1895, from Japan. Oka did not designate the type species of *Orobdella*. Soós (1966) subsequently designated *O*. *whitmani* as the type species by "position precedence" (Richardson 1971), because this species was cited first in the original description.

Oka (1895) also noted that *Orobdella* species were divided into three types on the basis of the mid-body somite annulation: 1) quadrannulate, *viz*., one somite (segment) consisting of four annuli (rings), species *O*. *whitmani*; 2) sexannulate (six annuli) species *O*. *ijimai*; and 3) octannulate (eight annuli) species *O*. *octonaria*. *Orobdella whitmani* was erected based on 18 specimens from Mt. Kinkazan, Gifu (Kinkwazan near Gifu in Oka's study), Mt. Kodaijiyama, Kyoto (Chi-on-in in Kiōto), and Mt. Kimposan, Kumamoto (Kibōzan near Kumamoto). *Orobdella ijimai* was described on the basis of ten specimens collected from Nikko, Tochigi (Nikkō). This species was later recorded from Amami-oshima Island in the Ryukyu Islands, Japan (Oka 1910a, b). Octannulate *O*. *octonaria* was described based on three materials from Mt. Kinkazan, and the Hakone mountain range, Kanagawa (the Hakone mountains). For all three species there was neither a sufficient description of their internal anatomy nor type designations. Thus, all of the *Orobdella* specimens in the study had been automatically considered syntypes of each species, in accordance with Article 73.2 of the International

Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). Oka mentioned that *Orobdella* specimens were deposited in the Zoological Institute of Tokyo Imperial University, which now forms part of the holdings of the Zoological Collection of The University Museum, The University of Tokyo (UMUTZ) (Nakano & Itoh 2011). In addition, some of the Oka's leech collections are now deposited in the National Museum of Nature and Science, Tokyo (NSMT) (Nakano 2010). To ensure sufficient morphological descriptions of these three species, the *Orobdella* specimens used in Oka (1895) had to be identified from the collection deposited in these museums, and reexamined.

Prior to the 1960s, only three species of *Orobdella* described by Oka (1895) had been known, and were recorded only from Japan. Gilyarov *et al.* (1969) later reported *O. whitmani* from Primorsky Krai in Far East Russia. On the basis of figure 1 in their paper, Nakano (2012c) believed that there was a strong possibility that Giryarov *et al*. misidentified the specimens, and the *Orobdella* species in Primorsky Krai may be an undescribed species. In the 1970s, an Australian hirudinologist L. R. Richardson examined *Orobdella* specimens from Japan (Richardson 1971, 1975). He redescribed *O. octonaria* on the basis of a non-type specimen from Nagano Prefecture, Japan, without subsequent type designation (Richardson 1971). In his paper, he erected a new genus *Kumabdella* Richardson, 1971 for *O. octonaria* principally according to its octannulate mid-body somites, and mentioned that the genus *Orobdella* should only include the quadrannulate species, *O. whitmani*, with another genus established for the sexannulate *O. ijimai*. However, his classification was not followed by subsequent taxonomic works (Lukin 1976; Sawyer 1986). Richardson (1975) then described one new quadrannulate *Orobdella* species, *O. kawakatsuorum* Richardson, 1975 from Hokkaido, Japan, of which the holotype has been deposited at NSMT. In his two papers, Richardson described the specimens' internal organs, and mentioned that *O. octonaria* and *O. kawakatsuorum* have a unique mid-gut organ (the gastroporal duct) and a euthylaematous pharynx.

In the early 2010s, our knowledge and understanding of the species diversity of *Orobdella* were advanced. Nakano (2010) discovered some of the syntypes of *O. whitmani* from Mt. Kinkazan at UMUTZ. Because specimens from its three syntype localities were determined to include two different species, he redescribed *O. whitmani* by designating one of the syntypes as a lectotype for this species, and also described one

new quadrannulate species *O. esulcata* Nakano, 2010 from Mt. Kimposan. He also revealed that both two species were gastroporous and euthylaematous pharyngeal species.

Nakano (2011b) erected a quadrannulate species *O. tsushimensis* Nakano, 2011 from Tsushima Island, Japan. Nakano (2011a) collected sexannulate *Orobdella* leeches from Nikko where is the type locality of *O. ijimai*, Amami-oshima Island, and additionally Okinawajima Island in the Ryukyu Islands to reevaluate the morphological characteristics of *O. ijimai*. Following an examination of the specimens, he revealed that sexannulate leeches in the Ryukyu Islands did not belong to *O. ijimai*. Therefore, he redescribed *O. ijiami* and described two new sexannulate species, *O. dolichopharynx* Nakano, 2011 from Amami-oshima Island, and *O. shimadae* Nakano, 2011 from Okinawajima Island, respectively. He also noted that no syntypes had been deposited at UMUTZ and NSMT. Nakano (2011a, b) described the four species as possessing a gastroporal duct and a euthylaematous pharynx in their internal organs.

Nakano (2012c) described a new quadrannulate species *O. koikei* Nakano, 2012, which is a gastroporous and euthylaematous pharyngeal leech, from Hokkaido. He also redescribed *O. kawakatsuorum* on the basis of the holotype and newly collected materials. Nakano (2012a) described a new sexannulate, gastroporous and euthylaematous pharyngeal species *O. mononoke* Nakano, 2012 from Yakushima Island in the Ryukyu Islands. Nakano (2012b) discovered a syntype of *O. octonaria*, which was collected from Hakone, at NSMT, and redescribed this species by designating the syntype as the lectotype. Furthermore, Nakano & Lai (2012) established a new quadrannulate species, *O. ketagalan* Nakano & Lai, 2012, which is also a gastroporous and euthylaematous pharyngeal species, from Taiwan. This was the first record of the genus *Orobdella* from Taiwan. Nakano & Seo (2012) reported *O. tsushimensis*, which is the first recorded species of *Orobdella* from South Korea. This specimen was collected from Gageodo Island, a small island southwest of the Korean Peninsula.

These studies revealed that at least eleven described and one undescribed species currently belong to the genus *Orobdella*, and are distributed in the Far East, *viz*., Japan, South Korea, Taiwan, and the Russian Far East (see Table 1 for the eleven described species). All of the eleven described species are gastroporous and euthylaematous pharyngeal leeches. A mid-gut organ, the gastroporal duct, is unique to the limited taxa in the Hirudinida, and has been reported from several species of African and Asian

leeches (Sawyer 1986). The gastroporal duct of *Orobdella* has two openings: one opening pore (the gastropore), is situated in the ventral body wall in front of the female gonopore; and another one, in the anterior ventral surface of a crop (*e.g*. Nakano (2012b)). These eleven *Orobdella* leeches consist of six quadrannulate species, four sexannulate species, and one octannulate species. Recently, several molecular phylogenetic studies regarding species relationships in this genus were conducted (Nakano 2012a, c; Nakano & Lai 2012; Nakano & Seo 2012; Nakano *et al.* 2012). These studies have revealed that sexannulation evolved in parallel in *Orobdella*, and the octannulate *O. octonaria*, which is the type species of *Kumabdella*, should definitely be included in a clade of mostly quadrannulate species. Nakano (2012b) and Nakano *et al.* (2012) concluded that *Kumabdella* should be considered a subjective junior synonym of *Orobdella* as per the classification in previous studies.

The systematic position of the genus *Orobdella* was controversial. Before revealing that this genus can be diagnosed by a gastroporal duct, *Orobdella* was placed in the family Erpobdellidae (Oka 1895; Soós 1966). Richardson (1971) revealed that *O. octonaria* is a gastroporous species, and established a new family Gastrostomobdellidae, in which he included *Orobdella* along with the genus *Gastrostomobdella* Moore, 1929. *Gastrostomobdella* is also a terrestrial and macrophagous taxon distributed in Southeast Asia and Hawaii, and possesses a gastroporal duct and euthylaematous pharynx (Moore 1929, 1935, 1946; Richardson 1971). In the original description of the family, Richardson noted that Gastrostomobdellidae belonged to the order Arhynchobdellida, but its relative family could not be determined. Sawyer (1986) revised the arhynchobdellid classification, and divided this order into two suborders, euthylaematous pharyngeal Hirudiniformes, and strepsilaematous pharyngeal Erpobdelliformes. In accordance with gastorstomobdellid leeches possessing a euthylaematous pharynx, he placed this family as a subfamily of the Central and South American macrophagous leech family Cylicobdellidae under Hirudiniformes. He also included the genus *Mimobdella* Blanchard, 1897 in Gastrostomobdellidae (Gastrostomobdellinae in his work). The genus *Mimobdella* was established with two new species (Blanchard 1897). Nakano (2011c) reexamined the holotype of the type species of *Mimobdella*, *M. japonica* Blanchard, 1897, and revealed that this species possesses a strepsilaematous pharynx. Thus, the genus *Mimobdella* clearly does not belong to the family Gastrostomobdellidae.

Oceguera-Figueroa *et al.* (2011) estimated the systematic position of Gastrostomobdellidae within the order Arhynchobdellida based on the molecular phylogenetic analyses. They revealed that *O. octonaria* was definitely included in the suborder Erpobdelliformes and concluded that Gastrostomobdellidae should be placed under Erpobdelliformes. But their molecular phylogenies did not include samples of *Gastrostomobdella*, the gastrostomobdellid type genus. Nakano *et al.* (2012) conducted molecular phylogenetic analyses to determine the phylogenetic position of *Orobdella* and *Gastrostomobdella*. Their phylogenetic analyses included all of the known *Orobdella* species at that time and a *Gastrostomobdella* leech. Their phylogenetic analyses revealed that *Orobdella* and *Gastrostomobdella* were rightfully placed within Erpobdelliformes, but *Orobdella* did not form a monophyletic clade with *Gastrostomobdella*. Then they erected the monotypic family Orobdellidae for the genus *Orobdella* on the basis of its morphology, especially that of a gastroporal duct, and the results of their phylogenetic analyses. Their classification is followed here. In addition, they undertook a reconstruction analysis to reveal the ancestral states of the pharynx in Erpobdelliformes, and revealed that a euthylaematous pharynx is a plesiomorphic character of the suborder. They concluded that Sawyer's systematic treatment of the morphology of a pharynx, as the erpobdelliform diagnostic character, should be reconsidered.

In this thesis, a systematic revision of the eleven known species of the monotypic family Orobdellidae is presented. In addition, their phylogenetic relationships are estimated using mitochondrial COI, and mitochondrial $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$, and 16S rRNA ($tRNA^{Cys}$–16S) sequence data.

Materials and methods

Taxon sampling and morphological analysis

Newly 107 *Orobdella* leeches were collected from 52 localities in Japan, under rocks or fallen leaves along mountain trails. Altitudes and coordinates for localities were obtained using a Germin eTrex® GPS unit. Addition to these newly collected materials, type specimens of the three *Orobdella* species, *O. kawakatsuorum* (holotype: NSMT-An 53), *O. octonaria* (lectotype: NSMT-An 415) and *O. whitmani* (lectotype: UMUTZ-Ann-Hir-5-1), and *Orobdella* specimens, which were used in previous studies (Nakano 2010, 2011a, b, 2012a, b, c; Nakano & Lai 2012; Nakano & Seo 2012) and have been deposited in the Zoological Collection of Kyoto University (KUZ), were examined. The detailed localities of all examined materials are shown in Figure 1. The morphologically examined specimens in this study are listed in Appendix.

The newly collected specimens were relaxed by the gradual addition of 99% ethanol to fresh water. For DNA extraction, botryoidal tissue was taken from the posterior part of the body around the caudal sucker of every specimen, and the rest of the bodies were fixed in 10% formalin and preserved in 70% ethanol. Two measurements were taken: body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker, and maximum body width (BW). Examination, dissection, and drawing of the specimens were performed under stereoscopic microscopes (LEICA S6E, M125 equipped with a drawing tube, and WILD HEERBRUGG TYP 308700 with a drawing tube). Newly collected specimens have been deposited in the KUZ.

The numbering convention is based on Moore (1927): body somites are denoted by Roman numerals, and the annuli in each somite are given an alphanumeric designation.

PCR and DNA sequencing

Extraction of genomic DNA and sequencing of the mitochondrial COI gene were done according to Nakano (2012c). Mitochondrial COI and $tRNA^{Cys}$–16S sequences of 56 *Orobdella* specimens and the $tRNA^{Cys}$–16S sequence of the holotype of *O. ketagalan* were newly obtained. In addition to these, COI and $tRNA^{Cys}$–16S sequences of 28 specimens of *Orobdella*, those of three other species of Erpobdelliformes, and the COI sequence of the holotype of *O. ketagalan* were obtained from GenBank (Table 2).

Phylogenetic analyses

Sequences of COI were aligned by eye because there were no indels. Sequences of $tRNA^{Cys}$–16S were aligned using MAFFT L-INS-i (Katoh *et al.* 2005) and then refined with GBLOCKS (Castresana 2000). The length of the aligned COI sequences was 1266 bp, and that of the aligned $tRNA^{Cys}$–16S was 737 bp (426 characters were eliminated). The concatenated sequences thus yielded a total of 2003 bp positions. Pairwise comparisons of Kimura-2 parameter (K2p) distance (Kimura 1980) of the COI sequences were calculated using MEGA5 (Tamura *et al.* 2011).

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) models. ML phylogenies were calculated using TREEFINDER v October 2008 (Jobb *et al.* 2004) with the tool package PHYLOGEARS v 2.0 (Tanabe 2008), and then nonparametric bootstrapping (Felsenstein 1985) was conducted with 500 replicates. The best-fit models for each partition were selected on the Akaike Information Criterion (Akaike 1974) by using KAKUSAN4 (Tanabe 2011): for the 1st position of COI, the transition model (TIM) with gamma distribution (+G) and proportion of invariant sites (+I) was selected; for the 2nd position, TIM+I; for the 3rd position, TIM+G; and for $tRNA^{Cys}$–16S, the general time reversal model (GTR)+G+I was selected. BI and Bayesian posterior probabilities (BPPs) were estimated using MPI version of MRBAYES v 3.2 (Altekar *et al.* 2004; Ronquist *et al.* 2012). The best-fit models for each partition were identified under the Bayesian information criterion (Schwarz 1978) and also by using KAKUSAN4: for the COI 1st position, GTR+G+I; for the COI 2nd position, Felstein 1981 model (F81)+I; for the COI 3rd position, GTR+G; and for $tRNA^{Cys}$–16S, GTR+G. Two independent runs of four Markov chains were conducted for 4 million generations, and tree was sampled every 100 generations. The parameter estimates and convergence were checked using TRACER v 1.5 (Rambaut and Drummond 2009). Based on these results, the first 10,001 trees were discarded.

Nodes with bootstrap (BS) values higher than 70% were considered as sufficiently resolved (Hillis and Bull 1993). Nodes with BPPs higher than 95% were considered statistically significant (Leaché and Reeder 2002).

Results

Systematics

Family **Orobdellidae** Nakano, Ramlah & Hikida, 2012

Gastrostomobdellidae Richardson, 1971: 588 (type genus *Gastrostomobdella* Moore, 1929; in part); Sawyer (1986): 678 (in part).
Orobdellidae Nakano *et al*., 2012: 183.

Diagnosis. Body firm, muscular, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker. Caudal sucker ventral, oval, its diameter slightly less than BW. Mid-body somites, quadr-, sex-, or octannulate. Post-anal annulus absent. Papillae numerous, minute, hardly visible, one row on every annulus. Pharynx agnathous, euthylaematous. Gastropore in anterior part of XIII, or absent. Gastroporal duct basically tubular, lying on female organ, canal of duct unbranched. Male gonopore at posterior part of XI. Female gonopore at anterior part of XIII. Gonopores separated by at least one full somite. Testisacs multiple, two or three in each annulus. Ejaculatory bulbs absent. Male medial reproductive system in posterior part of XI, being muscularized atrium, without penis sheath or penis. Ovisacs globular. Oviducts short, converging into common oviduct. Female median reproductive system essentially lacking.

Remarks. The molecular phylogeny in Nakano *et al.* (2012) showed that the genus *Orobdella* did not form a monophyletic clade with *Gastrostomobdella* (Fig. 2). The macrophagous leech family Orobdellidae can be distinguished from Gastrostomobdellidae by the following combination of characteristics: post-anal annulus absent (present in Gastrostomobdellidae); morphology of gastropore and gastroporal duct (see below); and ovisacs globular (elongated in Gastrostomobdellidae). The morphology of a gastropore and a gastroporal duct of *Orobdella* are quite different from those of *Gastrostomobdella* as follows: gastroporal duct basically tubular and lying on female organ (columnar and running vertically to gastropore in *Gastrostomobdella*); one pore opening to crop, canal of duct unbranched (two pores opening to crop, canal of duct Y-shaped); not penetrated through by nerve cord (penetrated through by nerve cord); and gastropore opening at XIII, in front of female gonopore (opening at XIV or XIV/XV, separated from female gonopore by at least

seven annuli) (Fig. 3).

Type genus. *Orobdella* Oka, 1895.

Additional genera. None. Monotypic.

Genus ***Orobdella*** Oka, 1895

Orobdella Oka, 1895: 278, 279; Soós (1966): 377, 381, 382; Richardson (1975): 42; Lukin (1976): 463, 464: Nakano (2010): 881; Nakano (2011a): 3.

Kumabdella Richardson, 1971: 590, 591 (type species, *Orobdella octonaria* Oka, 1895).

Diagnosis. Same as for the family Orobdellidae.

Etymology. The generic name is a feminine compound noun derived from the Greek words transliterated into Latin, oro (mountain), and bdella (leech), referring that leeches in this genus inhabit in mountainous regions (Oka 1895).

Remarks. Lukin (1976) and Sawyer (1986) synonymized the generic name *Kumabdella* subjectively with *Orobdella*. Their synonymizations were followed by the subsequent taxonomic, or phylogenetic studies (see details in Nakano (2012b) and Nakano *et al.* (2012)).

Type species. *Orobdella whitmani* Oka, 1895 (subsequent designation by Soós (1966)).

Additional species. *Orobdella dolichopharynx* Nakano, 2011, *O. esulcata* Nakano, 2010, *O. ijimai* Oka, 1895, *O. kawakatsuorum* Richardson, 1975, *O. ketagalan* Nakano & Lai, 2012, *O. koikei* Nakano, 2012, *O. mononoke* Nakano, 2012, *O. octonaria* Oka, 1895, *O. shimadae* Nakano, 2011, *O. tsushimensis* Nakano, 2011.

Orobdella whitmani Oka, 1895
(Figs 4, 5A, B, 6)

Orobdella whitmani Oka, 1895: 282–284 (in part), pl. 28, figs. 1, 4–8, pl. 29, fig. A, pl. 30, figs. 1, 3, 4; Oka (1910a): 19 (in part); Oka (1910b): 178 (in part); Soós (1966): 397; Lukin (1976): 464–466 (in part); Sawyer (1986): 680, 747; Nakano (2010): 882–884, figs. 3–5.

Diagnosis. Somite IV basically uniannulate. Somites VIII–XXV quadrannulate. Somite XXVI basically quadrannulate. Pharynx reaching to XIV. Gastropore conspicuous basically in middle of XIII b2. Gastroporal duct bulbous, winding at junction with gastropore. Male gonopore in middle of XI b6, female gonopore inconspicuous in middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli. Paired epididymides in middle part of XV to posterior part of XVIII, occupying 6–11 annuli. Ejaculatory ducts coiled, or straight in position anterior to ovisacs. Atrial cornua muscular, ovate.

Type specimens. Lectotype: UMUTZ-Ann-Hir-5-1 (designated by Nakano (2010)).

Type locality. Mt. Kinkazan (coordinates of the crest: 35°26′02″N, 136°46′56″E), Gifu, Gifu Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 4), maximum BL 117.5 mm (KUZ Z608), BW 7.6 mm (KUZ Z191), minimum BL 21.2 mm (KUZ Z527), BW 1.9 mm (KUZ Z603). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface generally greenish gray, yellow, or greenish brown, dorso-lateral surface yellow, or amber, ventral surface whitish yellow (Fig. 5A, B). Color faded in preservative, frequently with one dorsal dark line from II–XI b5/b6 to XXVI–caudal sucker; KUZ Z191, Z571 dark line reaching only to X b5–XI b5; or occasionally without any dark lines.

Somite I completely merged with prostomium (Fig. 6A). Somites II, III uniannulate (Fig. 6A); KUZ Z41, Z581, Z607 slight dorsal furrow present in III. Somite IV almost always uniannulate (Fig. 4A), rarely with one slight dorsal furrow; KUZ Z543, Z580 biannulate. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 6A, B). Somites VI, VII triannulate, a1 = a2 = a3 (Fig 6A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 6A–E); KUZ Z606 XVIII b6 with slight ventral furrow, b6 of each somite of XIV–XXIII with slight furrow, b6 of each somite of XXIV, XXV with slight dorsal furrow; KUZ Z607 every annulus, especially b6, of each somite of XIV–XXV with slight furrow; b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z191). Somite XXVI generally quadrannulate, a1 = a2 = a5 = a6, a1 = a2 > b5 = b6, a1 = a2 > b5 > b6, or a1 > a2 > b5 > b6 (Fig. 6C, D); rarely dorsally quadrannulate, a1 = a2 = b5 = b6, a1 = a2 > b5 = b6,

or a1 > a2 > b5 = b6, ventrally triannulate, a1 = a2 = a3, a1 = a2 < a3, or a1 > a2 < a3; scarcely ever triannulate, a1 = a2 < a3, or a1 > a2 < a3, generally with slight dorsal furrow in a3; last annulus of XXVI (generally b6, or occasionally a3) being ventrally last complete annulus (Fig. 6D). Somite XXVII almost always biannulate; or scarcely ever uniannulate; anus behind it with no post-anal annulus (Fig. 6C).

Anterior ganglionic mass almost always in VI a2 and a3; or scarcely ever in VI a1 and a2; KUZ Z608 in VI a3 and VII a1. Ganglion VII frequently in a2; rarely in a1 and a2; or scarcely ever in a1. Ganglia VIII, IX, of each somite, almost always in a2; or scarcely ever in a2 and b5; KUZ Z40 in a1 and a2. Ganglion X almost always in a2; or scarcely ever in a2 and b5. Ganglion XI generally in a2; or rarely in a2 and b5 (Fig. 6H). Ganglion XII often in a2; or occasionally in a2 and b5 (Fig. 6H); KUZ Z22 in a1; KUZ Z573 in b5; KUZ Z529 in b5 and b6. Ganglion XIII generally in a2 and b5 (Fig. 6H); or scarcely ever in a2, or in b5. Ganglia XIV, XV, of each somite, almost always in a2; or scarcely ever in a2 and b5 (Fig. 6H). Ganglia XVI–XVIII, of each somite, in a2 (Fig. 6H); KUZ Z27 in a1 and a2. Ganglion XIX almost always in a2; or scarcely ever in a1 and a2; KUZ Z 613 in a2 and b5. Ganglion XX generally in a2; or rarely in a1 and a2; KUZ Z40 in a2 and b5. Ganglion XXI often in a2; occasionally in a1 and a2. Ganglion XXII frequently in a2; or rarely in a1 and a2; KUZ Z607 in a1. Ganglion XXIII generally in a2; or rarely in a1 and a2. Ganglion XXIV in a1 and a2; or in a2. Ganglion XXV often in a1 and a2; rarely in a2; or scarcely ever in a1. Ganglion XXVI generally in a1; or scarcely ever in XXV b6 and XXVI a1; KUZ Z160, Z580, Z601 in XXV b6. Posterior ganglionic mass occasionally in XXVI a2–a3 (or b5, b6); in XXVI a3 (or b5, b6); or rarely in XXVI a3 (or b5, b6)–XVII; KUZ Z601 in XXVI a1 and a2; KUZ Z160 in XXVI a1–a3.

Eyes almost always in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 6A); rarely in one pair, without second and third pairs; KUZ Z607 in two pairs, lacking first pair; KUZ Z163–Z164, Z577, Z583 additional eyes present, one eye dorso-right on anterior part of III (KUZ Z577), one (KUZ Z164, Z165) or two eyes (KUZ Z163) on dorso-right of IV, or one additional pair dorsally on IV. Nephridiopores in 17 pairs in VIII–XXIV (Fig. 6B, E); or in 19 pairs in VIII–XXVI; situated ventrally at posterior margin of a1 of each somite. Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV a1–XIV/XV (Fig. 6F). Crop

tubular, reaching to XX a1/a2–XXI b6. Gastropore conspicuous, ventral, in middle of XIII a1; or slightly posterior to middle of XIII a1 (Fig. 6E, G). Gastroporal duct often bulbous, winding at junction with gastropore (Fig. 6F); rarely tubular, but slightly bulbous at junction with crop, winding at junction with gastropore; scarcely ever tubular, thick, winding at junction with gastropore; or scarcely ever simple tubular, narrow; joining with crop in XIV a1–XV a2 (Fig. 6F). Intestine tubular and acecate, reaching to XXIII a1/a2–XXIV b6. Rectum tubular, thin-walled.

Male gonopore in middle of XI b6; or slightly posterior to middle of XI b6 (Fig. 6E); KUZ Z527, Z583, Z584 in furrow of XI/XII. Female gonopore in middle of XIII a1 (Fig. 6E); or occasionally slightly posterior to middle of XIII a1; inconspicuous, located posterior to gastropore (Fig. 6G). Gonopores separated by 1/2 + 4 + 1/2 annuli (Fig. 6E); KUZ Z527, Z583, Z584 by 4 + 1/2. Testisacs multiple, in XVII a2/b5–XIX a1/a2 to XXIV b5–XXV b5/b6. Paired epididymides in XV a2/b5–XVI b6 to XVII a1/a2–XVIII/XIX (Fig. 6H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 (KUZ Z609 b6) to XV a2/b5–XVI b6; often coiled in position anterior to ovisacs; or occasionally straight in position anterior to ovisacs; coiled in position to posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 6H); KUZ Z584 with pre-atrial loop reaching to middle of XI b5. Pair of muscular atrial cornua, almost always ovate (Fig. 6H–K); KUZ Z572, Z584 fusiform; in XI b5 and b6 (Fig. 6H); KUZ Z609 in XI b6. Atrium short, muscular, globular, frequently in XI b6 (Fig. 6H–K); or rarely in XI b5 and b6. Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, generally in XIII a2 and b5 (Fig. 6H, L); or rarely in XIII a2; KUZ Z602 in XIII a2–b6. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a2 (Fig. 6H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 6L).

Distribution. Known from mountainous regions in the central part and the Chugoku Region of Honshu, and the northern part of Shikoku, Japan.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Professor C. O. Whitman, who was employed as the second Professor of Zoology at the University of Tokyo in 1879–1881.

Remarks. *Orobdella whitmani* possesses nephridiopores in 17, or 19 pairs. The

characteristic of 19 pairs of nephridiopores was observed only in the specimens from the Kinki Region in Honshu. The length of the epididymides of this species is also variable. The specimens from the central part of Honshu have epididymides in XV a2/b5–XVI a2 to XVII b5/b6–XVIII/XIX, which occupy 8–10 annuli. The epididymides of the specimens from the Kinki Region, the Chugoku Region and Shikoku are located in XV a2/b5–XVI b6 to XVII a1/a2–XVIII a2/b5, and occupy 6–8 annuli.

In the original description, Oka (1895) credited "Ijima MS" as the author of the specific name *whitmani*. Isao Ijima was the second Japanese professor of zoology at the Tokyo Imperial University (now The University of Tokyo), and reported the oogenesis of leeches (Ijima 1882; Nakano and Itoh 2011). Oka dedicated the authorship of this specific name to the memory of Professor Ijima. However, in accordance with Article 50 of the Code (International Commission on Zoological Nomenclature 1999), Oka should be credited this name, because it is clear that Oka alone is responsible for satisfying the criteria of the availability of the name *whitmani*.

Orobdella dolichopharynx Nakano, 2011
(Figs 5C, 7, 8)

Orobdella ijimai: Oka (1910a): 19 (in part); Oka (1910b) 178 (in part).
Orobdella dolichopharynx Nakano, 2011a: 6, 7, 10, figs. 5–7.

Diagnosis. Somite III uniannulate. Somite IV biannulate. Somite VI triannulate. Somite VII basically quadrannulate. Somite VIII basically quinquannulate. Somites IX–XXV sexannulate. Somite XXVI basically sexannulate. Pharynx reaching to XVI. Gastropore absent. Gastroporal duct rudimentary, thin-walled, tubular, but slightly bulbous at female gonopore, not joining with crop, reaching basically to XVI. Male gonopore basically in XI c11/c12, or in anterior margin of XI c12, female gonopore in XIII b1/b2, or in anterior margin of XIII b2, gonopores separated by 8 annuli. Epididymides absent. Sperm ducts coiled in position anterior to ovisacs with pre-atrial loop extending to middle of XI a2 to XI a2/b5. Atrial cornua absent.

Type specimens. Holotype: KUZ Z120 (Fig. 7).

Type locality. Mt. Yuwandake, alt. 448 m, 28°17′11″N, 129°18′56″E, Uken, Amami-oshima Island, Kagoshima Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 7), maximum BL 112.4 mm, BW 7.5 mm (KUZ Z119), minimum BL 58.9 mm, BW 3.5 mm (KUZ Z117). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface blackish green, or greenish brown, dorso-lateral surface yellow, or yellowish white, ventral surface yellow or yellowish white (Fig. 5C). Color faded in preservative, without any dark lines.

Somite I completely merged with prostomium (Fig. 8A). Somites II, III uniannulate (Fig. 8A); KUZ Z115 slight dorsal furrow present in III. Somite IV biannulate (Fig. 8A); KUZ Z115 slight dorsal furrow present in (a1 + a2). Somite V biannulate, (a1 + a2) > a3; KUZ Z115 slight dorsal furrow present in (a1 + a2); a3 forming posterior margin of oral sucker (Fig. 8A, B). Somite VI triannulate, a1 = a2 = a3 (Fig. 8A, B). Somite VII generally quadrannulate, a1 = a2 > b5 = b6 (Fig. 8A, B); KUZ Z124, Z567, Z582 triannulate, a1 = a2 < a3 frequently slight dorsal furrow present in a3. Somite VIII quinquannulate, a1 > a2 = b5 > c11 = c12 (Fig. 8A, B), KUZ Z115, Z117, Z123 slight dorsal furrow present in a1. Somites IX–XXV sexannulate (Fig. 8A–E), anterior part of somites, b1 = b2 < a2 = b5 > c11 = c12; middle and posterior part of somites, b1 = b2 = a2 = b5 = c11 = c12; b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z118, Z120, Z121). Somite XXVI often sexannulate, b1 = b2 = a2 = b5 > c11 = c12; or occasionally quinquannulate, annulation hardly decidable, possibly a1 = a2 = b5 < c11 > c12, or b1 = b2 < a2 > b5 = b6; KUZ Z122 quadrannulate, a1 = a2 = b5 < b6; last annulus of XXVI (often c12, or b6) being ventrally last complete annulus (Fig. 8C, D). Somite XXVII biannulate; or quadrannulate; KUZ Z116, Z122 uniannulate; anus behind it with no post-anal annulus (Fig. 8C).

Anterior ganglionic mass almost always in VI a2 and a3; KUZ Z567 in VI a1 and a2. Ganglion VII generally in a2; KUZ Z567 in a1; KUZ Z119 in a1 and a2. Ganglia VIII–XI, XIII–XV, XVII in a2 of each somite (Fig. 8H). Ganglia XII, XVI, XVIII, XX almost always in a2 (Fig. 8H); KUZ Z567 ganglia XII, XX in b2 and a2, ganglion XVI in a2 and b5; KUZ Z582 ganglion XVIII in b2 and a2. Ganglion XXI often in a2; or occasionally in b2 and a2. Ganglion XXII frequently in b2 and a2; KUZ Z121, Z129, Z582 in a2. Ganglion XXIII often in b2 and a2; KUZ Z121, Z122, Z129 in a2; KUZ Z119 in b2. Ganglion XXIV generally in b2 and a2; KUZ Z129 in a2 and b5; KUZ

Z582 in b2. Ganglion XXV occasionally in b2; or in b2 and a2; KUZ Z129 in a2. Ganglion XXVI located variably; KUZ Z116, Z118, Z120 in a1 and a2; KUZ Z119, Z567 in b1; KUZ Z129, Z582 in b2; KUZ Z122 in a1; KUZ Z121 in b1 and b2. Posterior ganglionic mass often in XXVI b5 to last annulus of XXVI (b6 or c12); KUZ Z567 in XXVI a2–c11; KUZ Z116 in XXVI a2–c12; KUZ Z119 in a2 and b5.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 8A). Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of VIII a1, and b2 of each somite of IX–XXIV (Fig. 8B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XVI b1–XVII b2/a2 (Fig. 8F). Crop tubular, reaching to XXII a2–XXIII b1/b2. Gastropore absent (Fig. 8E, G). Gastroporal duct rudimentary, thin-walled, tubular, but slightly bulbous at female gonopore, not joining with crop, reaching to XV b2/a2–XVI b6 (Fig. 8F). Intestine tubular and acecate, reaching to XXIII c12–XXV b5. Rectum tubular, thin-walled.

Male gonopore in anterior margin of XI c12 (Fig. 8E); or in furrow of XI c11/c12; KUZ Z129 in middle of XI c11. Female gonopore in furrow of XIII b1/b2; or in anterior margin of XIII b2 (Fig. 8E). Gonopores separated by almost always 8 annuli (Fig. 8E); KUZ Z129 1/2 + 8 annuli. Testisacs multiple, in XVII b5–XVIII b5 to XXV a2–XXV c11/c12 (Fig. 8H). Sperm ducts in XI a2 to XVII b5–XVIII b5; coiled in position anterior to ovisacs; hardly coiled in position posterior to ovisacs; each widening from respective junction with testisacs, narrowing at junction with atrium, with pre-atrial loop extending to middle of XI a2 to XI a2/b5 (Fig. 8H, I). Epididymides absent. Ejaculatory bulbs absent. Atrial cornua absent. Atrium short, muscular, globular, in XI c11 and c12 (Fig. 8H, I). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, almost always in XIII a2 (Fig. 8H, J); KUZ Z567 in XIII b2 and a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII b2 (Fig. 8H, J). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 8J).

Distribution. Known from mountainous regions in Amami-oshima Island, Kakeromajima Island, and Tokunoshima Island in the Ryukyu Islands, Japan.

Etymology. The specific name is a compound noun in apposition derived from the Greek words transliterated into Latin, dolicho (long), and pharynx (pharynx), referring

that this species possesses a longer pharynx as compared to the other *Orobdella* species except for *O. shimadae* (Nakano 2011a).

Remarks. In the original description (Nakano 2011a), a clitellum of this species was described as being in X a2–X b5 to XIII a2–XIV b1. Following a reexamination of the specimens used in Nakano (2011a), a clitellum of *O. dolichopharynx* was found to be located from X b5 to XIII a2. The description in Nakano (2011a) was thus emended as described above.

The specimen KUZ Z582 from Kakeromajima Island possesses a short gastroporal duct that reaches to XV b2/a2. The other specimens have a longer gastroporal duct that reaches to XVI b1 to XVI b6. This short gastroporal duct is a diagnostic character of *O. shimadae* from Okinawajima Island as described below. However, the specimen from Kakeromajima Island was identified as *O. dolichopharynx* based on its possession of the other diagnostic characters of the species. The molecular phylogenetic analyses revealed that the specimen from Kakeromajima Island clearly belonged to *O. dolichopharynx* (see below).

The species identity of the specimen from Tokunoshima Island is also problematic. This specimen has a male gonopore in the middle part of XI c11 in contrast to a male gonopore situated in the furrow of XI c11/c12 or in the anterior margin of XI c12 in the specimens from Amami-oshima Island and Kakeromajima Island. The taxonomic position of the sexannulate *Orobdella* species from Tokunoshima Island should be reconsidered (see Discussion below).

Orobdella esulcata Nakano, 2010
(Figs 5D–F, 9, 10)

Orobdella whitmani: Oka (1895): 282–284 (in part).
Orobdella esulcata Nakano, 2010: 884–886, fig. 6.

Diagnosis. Somite IV uniannulate. Somites VIII–XXV quadrannulate. Somite XXVI basically triannulate. Pharynx reaching to XIV. Gastropore conspicuous in middle of XIII a1. Gastroporal duct basically tubular, but slightly bulbous and winding at junction with gastropore. Male gonopore in middle of XI b6, female gonopore inconspicuous in middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli.

Paired epididymides in middle part of XV to middle part of XX, occupying 10–15 annuli. Ejaculatory ducts loosely coiled in position anterior to ovisacs. Atrial cornua muscular, ovate.

Type specimens. Holotype: KUZ Z29.

Type locality. Mt. Kimposan, alt. 412 m, 32°48′36″N, 130°38′29″E, Kumamoto, Kumamoto Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 9), maximum BL 97.1 mm (KUZ Z29), BW 6.0 mm (KUZ Z171), minimum BL 15.1 mm, BW 1.8 mm (TN954). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface bluish gray, brown, or dark brown, ventral purplish ash gray, yellowish gray, or reddish white (Fig. 5D–F). Color faded in preservative, frequently without any dorsal dark lines; KUZ Z590, Z595–Z597 with one dark dorsal line.

Somite I completely merged with prostomium (Fig. 10 A). Somites II–IV uniannulate (Fig. 10A); KUZ Z556 slight dorsal furrow present in IV. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 10A, B). Somites VI, VII triannulate, a1 = a2 = a3 (Fig. 10A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 10A–E); a2 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z590). Somite XXVI almost always triannulate, a1 = a2 = a3, a1 = a2 > a3, a1 > a2 < a3 (Fig. 10C, D), KUZ Z31 with slight furrow in a3; KUZ Z592, Z593 with slight dorsal furrow in a3; KUZ Z594, Z597 dorsally triannulate, a1 = a2 = a3, ventrally biannulate a1 < (a2 + a3); last annuls of XXVI (almost always a3, or scarcely ever (a2 + a3)) being ventrally last complete annulus (Fig. 10D). Somite XXVII sometimes uniannulate; rarely biannulate; or scarcely ever triannulate; anus behind it with no post-anal annulus (Fig. 10C).

Anterior ganglionic mass generally in VI a2 and a3; KUZ Z32 in VI a1–a3; KUZ Z598 in VI a2 and a3, and VII a1. Ganglion VII generally in a2; KUZ Z32, Z590 in a1 and a2; KUZ Z598 in a2 and a3. Ganglia VIII, X–XII of each somite, often in a2; or occasionally in a2 and b5 (Fig. 10H). Ganglion IX generally in a2; KUZ Z171, Z556, Z592 in a2 and b5. Ganglion XIII often in a2 and b5 (Fig. 10H); or occasionally in a2. Ganglion XIV frequently in a2; or rarely in a2 and b5 (Fig. 10H). Ganglion XV almost always in a2 (Fig. 10H); KUZ Z171 in a2 and b5. Ganglion XVI in a2 (Fig. 10H).

Ganglia XVII–XIX, of each somite, almost always in a2 (Fig. 10H); KUZ Z171 in a1 and a2. Ganglia XX–XXII generally in a2 of each somite; KUZ Z171, Z557, Z591 ganglion XX in a1 and a2; KUZ Z591 ganglion XXI in a1; KUZ Z557, Z592 ganglion in a1 and a2; KUZ Z170, Z557, Z593 ganglion XXII in a1 and a2. Ganglion XXIII frequently in a2; or rarely in a1 and a2. Ganglion XXIV often in a1 and a2; or occasionally in a2. Ganglion XXV frequently in a1 and a2; or rarely in a1; KUZ Z51 in a2. Ganglion XXVI occasionally in XXV b6; rarely in XXV b6 and XXVI a1; or rarely in XXVI a1; KUZ Z557 in XXV b5 and b6. Posterior ganglionic mass occasionally in XXVI a2 and a3; KUZ Z30, Z31, Z51 in XXVI a3.

Eyes almost always in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 10A); KUZ Z171, Z172 in one pair, without second and third pairs; KUZ Z591 in four pairs, additional one pair on III. Nephridiopores almost always in 17 pairs in VIII–XXIV (Fig. 10B, E); KUZ Z596 in 18 pairs in VIII–XXV; situated ventrally at posterior margin of a1 of each somite (Fig. 10B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV a1–b6 (Fig. 10F). Crop tubular, reaching to XIX/XX–XX/XXI. Gastropore conspicuous, ventral, frequently slightly anterior to middle of XIII a1 (Fig. 10E, G); or rarely in middle of XIII a1. Gastroporal duct often tubular, but slightly bulbous and winding at junction with gastropore (Fig. 10F); KUZ Z556, Z557, Z596, bulbous, slightly winding at junction with gasropore; KUZ Z171 tubular, but slightly bulbous at junction with crop; joining with crop in XIV a2–b6 (Fig. 10F). Intestine tubular and acecate, reaching to XXII b6–XXIV b6. Rectum tubular, thin-walled.

Male gonopore often in middle of XI b6; or occasionally slightly anterior to middle of XI b6 (Fig. 10E). Female gonopore frequently slightly anterior to middle of XIII a1 (Fig. 10E); or rarely in middle of XIII a1; inconspicuous, located posterior to gastropore (Fig. 10E, G). Gonopores separated by 1/2 + 4 + 1/2 annuli (Fig. 10E). Testisacs multiple, in XVIII a1/a2–XX a2/b5 to XXIV b6–XXVI a2 (Fig. 10H). Paired epididymides in XV a2/b5–XVII b5/b6 to XVIII a1/a2–XX b5 (Fig. 10H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 to XV a2/b5–XVII b5/b6; loosely coiled in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then

turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 10H). Pair of muscular atrial cornua, ovate, in XI b5 and b6 (Fig. 10H–K). Atrium short, muscular, globular, generally in XI b5 and b6 (Fig. 10H–K); KUZ Z590, Z596, Z598 in XI b6. Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, almost always in XIII a2 and b5 (Fig. 10H, L); KUZ Z591, Z592 in XIII a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII a2 (Fig. 10H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 10L).

Distribution. Known from mountainous regions in the northern part of Kyushu, Japan and peripheral small islands to Kyushu.

Etymology. The specific name is a feminine adjective derived from the Latin words, e (lacks), and sulcus (furrow), referring that this species possesses no annular furrow in somite IV and XXVI (Nakano 2010).

Remarks. Although most of the specimens of *O. esulcata* possess a tubular gastroporal duct that is slightly bulbous at the junction with a gastropore, morphological variation in the gastroporal duct was observed among the examined materials. Three specimens (KUZ Z556, Z557, Z596) have a completely bulbous gastroporal duct, and one specimen from Ikinoshima Island (KUZ Z171) bears a duct that is slightly bulbous at the junction with a crop. However, these quadrannulate specimens were identified as *O. esulcata* due to their possession of the following characteristics: triannulate XXVI; male gonopore in middle of XI b6; female gonopore in middle of XIII a1; epididymides that occupies more than 10 annuli; and ovate atrial cornua. Their species identity was also recovered by the present molecular phylogenies (see below).

Orobdella ijimai Oka, 1895
(Figs 11, 12A, 13)

Orobdella ijimai Oka, 1895: 284–285, pl. 28, figs. 2, 9, 10, pl. 29, fig. B, pl. 30, figs. 2, 5–8; Oka (1910a): 19 (in part); Oka (1910b): 178 (in part); Soós (1966): 397; Lukin (1976): 466; Sawyer (1986): 680, 747; Nakano (2011a): 3, 4, 6, figs. 2–4.

Diagnosis. Somite III basically biannulate. Somite IV biannulate. Somite VI triannulate. Somite VII quadrannulate. Somites VIII–XXV sexannulate. Somite XXVI basically

quadrannulate. Pharynx reaching to posterior part of XIII to XIII/XIV. Gastropore conspicuous, basically in middle of XIII b2. Gastroporal duct bulbous, winding at junction with gastropore. Male gonopore basically in middle of XI c12, female gonopore inconspicuous, basically in XIII b2, behind gastropore, gonopores separated by 1/2 + 7 + 1/2 annuli. Paired epididymides in posterior part of XVI to posterior part of XIX, occupying 11–17 annuli. Ejaculatory ducts nearly straight in position anterior to ovisacs. Pre-atrial loops absent. Atrial cornua muscular, ovate.

Type specimens. Syntypes: missing (Nakano 2011a).

Type locality. Nikko, Tochigi Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 11), maximum BL 95.1 mm (KUZ Z113), BW 6.4 mm (KUZ Z109), minimum BL 49.2 mm, BW 3.0 mm (KUZ Z112). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface blackish green, dorso-lateral surface yellow, ventral surface whitish yellow (Fig. 12A). Color faded in preservative, without any dark lines.

Somite I completely merged with prostomium (Fig. 13A). Somite II uniannulate (Fig. 13A). Somite III frequently biannulate (Fig. 13A); or rarely uniannulate. Somite IV biannulate (Fig. 13A). Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 13A, B). Somite VI triannulate, a1 = a2 = a3 (Fig. 13A, B). Somite VII quadrannulate, a1 = a2 = b5 = b6 (Fig .13A, B). Somites VIII–XXV sexannulate, b1 = b2 = a2 = b5 = c11 = c12 (Fig. 13A–E); b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z110). Somite XXVI often quadrannulate, a1 = a2 = b5 < b6 (Fig. 13C, D), KUZ Z550, 585 slight dorsal furrow present in b6, KUZ Z190 slight dorsal furrow present in b6; KUZ Z111, Z549 quinquannulate, a1 = a2 = b5 > c11 = c12; KUZ Z549 quinquannulate, b1 = b2 = a2 = b5 < b6, b6 with slight dorsal furrow; KUZ Z188, Z189 sexannulate, b1 = b2 = a2 = b5 = c11 = c12, b1 = b2 = a2 = b5 = c11 > c12; last annulus of XXVI (b6, or c12) being ventrally last complete annulus (Fig. 13D). Somite XXVII biannulate; or uniannulate (Fig. 13C); anus behind it with no post-anal annulus (Fig. 10C).

Anterior ganglionic mass in VI a1–a3; or in VI a2 and a3. Ganglion VII frequently in a2; KUZ Z188 in a2 and b5. Ganglion VIII often in a2 and b5; KUZ Z108, Z110, Z113 in a2. Ganglia IX, XII, of each somite, almost always in a2 (Fig. 13H); KUZ Z585

ganglion IX in a2 and b5; KUZ Z604 ganglion XII in a2 and b5. Ganglia X, XIV in a2 of each somite (Fig. 13H). Ganglion XI frequently in a2 and b5 (Fig. 13H); KUZ Z188, Z604 in a2. Ganglion XIII almost always in a2 and b5; KUZ Z108 in b5 (Fig. 13H). Ganglia XV–XVII, XIX, XXI, XXII of each somite, almost always in a2 (Fig. 13H); KUZ Z585 in b2 and a2. Ganglia XVIII, XXIV, of each somite, frequently in a2 (Fig. 13H); KUZ Z585, Z604 in b2 and a2. Ganglion XX often in b2 and a2; KUZ Z110, Z188, Z549 in a2. Ganglion XXIII often in a2; KUZ Z188, Z585, Z604 in b2 and a2. Ganglion XXV almost always in b2 and a2; KUZ Z113 in a2. Ganglion XXVI almost always in anterior annuli of XXVI (a1, or b1 and b2); KUZ Z188 in XXV c12 and XXVI b1. Posterior ganglionic mass frequently in posterior annuli of XXVI (b5 and b6, or b5 and c11); KUZ Z188, Z549 in middle annuli of XXVI (a2–b6, or b2–c11).

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig .13A). Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of b2 of each somite (Fig. 13B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIII c12–XIII/XIV (Fig. 13F). Crop tubular, reaching to XX a2/b5–XXI a2. Gastropore conspicuous, ventral, frequently in middle of XIII b2 (fig. 13E, G); or rarely in anterior of XIII b2. Gastroporal duct muscular, bulbous, winding at junction with gastropore, joining with crop in XIII/XIV–XIV a2 (Fig. 13F). Intestine tubular and acecate, reaching to XXIV b2/a2–XXV b1. Rectum tubular, thin-walled.

Male gonopore generally in middle of XI c12 (Fig. 13E); KUZ Z109, Z189, Z585 in anterior of XI c12. Female gonopore frequently in middle of XIII b2;or rarely in anterior of XIII b2; inconspicuous, located posterior to gastropore (Fig. 13G). Gonopores separated by 1/2 + 7 + 1/2 annuli (Fig. 13E). Testisacs multiple, in XVIII c11–XIX c11/c12 to XXV b1–XXV/XXVI (Fig. 13H). Paired epididymides in XVI b2/a2–XVIII b1/b2 to XVIII b2/a2–XIX c11 (Fig. 13H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 (KUZ Z113, Z549 c11) to XVI b2/a2–XVIII b1/b2; nearly straight in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 13H). Pair of muscular atrial cornua, almost always ovate (Fig. 13H–K); KUZ Z585 fusiform; frequently in XI b5– c12; KUZ Z113, Z549 in XI c11 and c12. Atrium short,

muscular, globular, in XI c11 and c12 (Fig. 13H–K). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, often in XIII a2 and b5; KUZ Z585 in XIII a2; KUZ Z110 in XIII a2–c11; KUZ Z108 in XIII b5 and c11 (Fig. 13H, L). Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct frequently in XIII a2 (Fig. 13H, L); KUZ Z585 XIII b2; KUZ Z549 in XIII b2/a2. Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 13L).

Distribution. Known from mountainous regions in the northern Kanto District in Honshu, Japan.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Professor I. Ijima.

Remarks. One specimen KUZ Z585 possesses fusiform atrial cornua. Because the testisacs of this specimen were difficult to detect, this specimen may be an immature leech. Mature individuals of *O. ijimai* bear ovate atrial cornua.

Nakano (2011a) provided an emendment to the original description of this species in Oka (1895) on the basis of the specimens from the type locality.

Orobdella kawakatsuorum Richardson, 1975
(Figs 12B, 14, 15)

Orobdella whitmani: Oka (1910a): 19 (in part); Oka (1910b) 178 (in part).
Orobdella kawakatsuorum Richardson, 1975: 42–51, figs. 1, 2; Nakano (2012c): 19–24, figs. 6–11.

Diagnosis. In life, dorsal surface navy blue. Somite IV basically biannulate. Somites VIII–XXV quadrannulate. Somite XXVI Pharynx reaching to XIV. Gastropore conspicuous in furrow of XIII a1/a2. Gastroporal duct simple tubular, thin-walled. Male gonopore basically in anterior margin of XI b6, female gonopore inconspicuous in furrow of XIII a1/a2, behind gastropore, gonopores separated by 6 annuli. Paired epididymides in middle part of XVI to posterior part of XVII, occupying 1–2 annuli. Ejaculatory ducts nearly straight, or loosely coiled in position anterior to ovisacs. Atrial cornua undeveloped, conical.

Type specimens. Holotype: NSMT-An 53.

Type locality. Home garden of Professor Masaharu Kawakatsu, Sapporo, Hokkaido, Japan (now has been destroyed, Kawakatsum pers. comm.).

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 14), maximum BL 111.6 mm (KUZ Z142), BW 6.77 mm (KUZ Z169), minimum BL 15.1 mm (KUZ Z149), BW 1.65 mm (KUZ Z187). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface navy blue, ventral surface purplish white, reddish ash gray, or yellowish ash gray (Fig. 12B). Color faded in preservative, without any dark lines.

Somite I completely merged with prostomium (Fig. 15A). Somite II uniannulate (Fig. 13A). Somite III often uniannulate, occasionally with slight dorsal furrow; or occasionally biannulate (Fig. 15A). Somite IV generally biannulate (Fig. 15A); or rarely uniannulate, generally with slight dorsal furrow. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 15A, B). Somites VI, VII triannulate, a1 = a2 = a3 (Fig. 15A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 15A–E); b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z24, Z143, Z148, Z168). Somite XXVI almost always triannulate, a1 = a2 = a3, rarely with slight dorsal furrow in a3 (Fig. 15C, D); KUZ Z143 dorsally triannulate, a1 = a2 = a3, ventrally biannulate, a1 < (a2 + a3); last annulus of XXVI (almost always a3, or scarcely ever (a2 + a3)) being ventrally last complete annulus (Fig. 15D). Somite XXVII often uniannulate (Fig. 15C); or occasionally biannulate; anus behind it with no post-anal annulus (Fig. 15C).

Anterior ganglionic mass occasionally in VI a2 and a3: or occasionally in VI a3 and VII a1; KUZ Z152, Z154 in VI a2 and a3, and VII a1; KUZ Z159 in VI a1–a3. Ganglion VII occasionally in a2; or occasionally in a3; KUZ Z141, Z148, Z169 in a2 and a3. Ganglion VIII occasionally in a2 and b5; KUZ Z24, Z143, Z159 in a2; KUZ Z152–Z154 in b5. Ganglion IX often in a2 and b5; KUZ Z24, Z141, Z169 in a2; KUZ Z150 in b5. Ganglion X occasionally in a2 and b5; or occasionally in a2; KUZ Z150, Z152 in b5. Ganglion XI almost always in a2 and b5 (Fig. 15H); KUZ Z24 in a2. Ganglion XII frequently in a2 and b5 (Fig. 15H); or rarely in a2. Ganglion XIII almost always in a2 and b5 (Fig. 15H); KUZ Z159 in b5. Ganglion XIV often in a2; or occasionally in a2 and b5 (Fig. 15H). Ganglion XV generally in a2 (Fig. 15H); KUZ Z141, Z143, Z159 in a2 and b5. Ganglia XVI, XX in a2 of each somite (Fig. 15H).

Ganglia XVII, XIX, XXI, of each somite, almost always in a2 (Fig. 15H); KUZ Z152 ganglia XVII, XIX in a2 and b5; KUZ Z153 ganglion XXI in a1 and a2. Ganglion XVIII generally in a2; KUZ Z150 in a1 and a2; KUZ Z152 in a2 and b5. Ganglion XXII frequently in a2; KUZ Z153, Z154, Z159 in a1 and a2. Ganglion XXIII in a2; or in a1 and a2. Ganglion XXIV in a1 and a2; or in a2; KUZ Z152 in a1. Ganglion XXV occasionally in a1; or occasionally in a1 and a2; KUZ Z24, Z148 in a2. Ganglion XXVI occasionally in XXV b6; or rarely in XXV b6 and XXVI a1; KUZ Z150, Z167 in XXVI a1; KUZ Z148 in XXVI a2. Posterior ganglionic mass sometimes in XXVI a2 and a3; or rarely in XXVI a1 and a2; KUZ Z143, Z159 in XXVI a1–a3.

Eyes often in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 15A); or rarely in one pair, without second and third pairs; KUZ Z169 hardly detectable. Nephridiopores almost always in 17 pairs in VIII–XXIV (Fig. 15B, E); NSMT-An 53, KUZ Z155 in 18 pairs in VIII–XXV; situated ventrally at posterior margin of a1 of each somite (Fig. 15B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV a2–XIV/XV (Fig. 15F). Crop tubular, reaching to XX b5/b6–XXI a1/a2. Gastropore conspicuous, ventral, almost always in furrow of XIII a1/a2 (Fig. 15E, G); KUZ Z168 in anterior margin of XIII a1. Gastroporal duct simple tubular, thin-walled, joining with crop in XIII a2–XIV/XV (Fig. 15F). Intestine tubular and acecate, reaching to XXI a1–XXIV b5. Rectum tubular, thin-walled.

Male gonopore almost always in anterior margin of XI b6; KUZ Z140, Z154, Z167 in furrow of XI b5/b6 (Fig. 15E). Female gonopore almost always in furrow of XIII a1/a2; KUZ Z168 in anterior margin of XIII a1; inconspicuous, located posterior to gastropore (Fig. 15G). Gonopores separated by 6 annuli (Fig. 15E). Testisacs multiple, in XVI b5/b6–XVII b6 to XXIII a1–XXV b6 (Fig. 15H). Paired epididymides in XVI a2/b5–XVII b5 to XVI b5/b6–XVII b6 (Fig. 15H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 to XVI a2/b5–XVII b5; nearly straight in position anterior to ovisacs; or loosely coiled in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning gradually inward toward atrial cornua without pre-atrial loop (Fig. 15H). Pair of atrial cornua, undeveloped, conical, almost always in XI b5 and b6 (Fig. 15H–K); KUZ Z24 in XI b5. Atrium short, muscular, globular, in XI b5 and b6

(Fig. 15H–K). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, almost always in XIII a2 and b5 (Fig. 15H, L); KUZ Z154 in XIII a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII a2 (Fig. 15H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 15L).

Distribution. Known from mountainous regions in Hokkaido, Japan.

Etymology. The specific name is a noun in the genitive case formed directly from the names of Dr. M. Kawakatsu and his family (Richardson 1975).

Remarks. Nakano (2012c) emended the original description of *O. kawakatsuorum* in Richardson (1975) based on the holotype and additional specimens.

Orobdella ketagalan Nakano & Lai, 2012
(Figs 16, 17)

Orobdella ketagalan Nakano & Lai, 2012: 53–58, figs. 2–4.

Diagnosis. Somite IV uniannulate. Somites VIII–XXV quadrannulate. Somite XXVI basically triannulate. Somite Pharynx reaching to posterior of XIV to anterior of XV. Gastropore conspicuous in middle of XIII a1. Gastroporal duct narrow, tubular. Male gonopore in middle of XI b6, female gonopore inconspicuous in middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli. Small paired sperm duct bulbs in XV, occupying 1–2 annuli. Epididymides absent. Ejaculatory ducts nearly straight in position anterior to ovisacs. Atrial cornua undeveloped, conical.

Type specimens. Holotype: KUZ Z208 (Fig. 16).

Type locality. Yangmingshan National Park, alt. 779 m, 25°11′07″N, 121°31′10″E, Taipei City, Taiwan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 16), maximum BL 111.7 mm, BW 10.3 mm (KUZ Z210), minimum BL 57.63 mm, BW 4.2 mm (KUZ Z211). Caudal sucker ventral, oval, its greater diameter smaller than BW. Color faded in preservative, generally without any dark lines; KUZ Z209 with one dorsal dark line from VI a2 to middle of XXVII.

Somite I completely merged with prostomium (Fig. 17A). Somites II–IV uniannulate (Fig. 17A). Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 17A, B). Somites VI, VII triannulate, a1 = a2 = a3 (Fig. 17A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6; b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z208–Z211) (Fig. 17A–E). Somite XXVI frequently triannulate, a1 = a2 = a3, a1 = a2 > a3, or a1 = a2 < a3 (Fig. 17C, D), KUZ Z197, Z207 a3 with slight dorsal furrow present; KUZ Z201, Z211 quadrannulate, a1 = a2 > b5 = b6; last annulus of XXVI (frequently a3, or rarely b5) being ventrally last complete annulus (Fig. 17D). Somite XXVII frequently biannulate; KUZ Z197, Z208 uniannulate (Fig. 17C); anus behind it with no post-anal annulus (Fig. 17C).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII frequently in a2; KUZ Z210 in a1. Ganglia VIII–X, XII, XIV–XXIII in a2 of each somite (Fig. 17H). Ganglion XI frequently in a2 (Fig. 17H); KUZ Z201 in a2 and b5. Ganglion XIII in a2 and b5 (Fig. 17H). Ganglion XXIV frequently in a1 and a2 (Fig. 17H); KUZ Z209 in a2. Ganglion XXV in a1 and a2; KUZ Z210 in a1. Ganglion XXVI frequently in XXV b6; KUZ Z209 in XXV b6 and XXVI a1. Posterior ganglionic mass frequently in XXVI a2 and a3 (or b5); KUZ Z208 in XXVI a1–a3.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 17A). Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of a1 of each somite (Fig. 17B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV a2/b5–XV a1 (Fig. 17F). Crop tubular, reaching to XIX a2–XXI/XXII. Gastropore conspicuous, ventral, in middle of XIII a1 (Fig. 17E, G). Gastroporal duct narrow, simple tubular, joining with crop in XIV b5–XIV/XV (Fig. 17F). Intestine tubular and acecate, reaching to XIV a2/b5–XXV a2. Rectum tubular, thin-walled.

Male gonopore in middle of XI b6 (Fig. 17E). Female gonopore in middle of XIII a1, inconspicuous, located posterior to gastropore (Fig. 17G). Gonopores separated by 1/2 + 4 + 1/2 annuli (Fig. 17E). Testisacs multiple, in XV a1/a2–XVI/XVII to XXIII a1–XXV b5 (Fig. 17H). Sperm ducts in XI b5 to XV a1/a2–XVI/XVII; loosely coiled in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua; with pre-atrial loop reaching to XI a2/b5 to middle of XI b5 (Fig. 17H, J); small paired bulbs

in XV a1–b5, each bulb occupying one annulus (Fig. 17H, I). Epididymides absent. Ejaculatory bulbs absent. Pair of atrial cornua undeveloped, conical, in XI b5 and b6 (Fig. 17H, J–L). Atrium short, muscular, globular, in XI b5 and b6 (Fig. 17H, I–L). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, in XIII a2 and b5 (Fig. 17H, M). Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a1/a2 (Fig. 17H, M). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 17M).

Distribution. Known from the type locality and adjacent areas in Yangmingshan National Park, Taiwan.

Etymology. The specific name is a native word taken from the aboriginal Taiwanese tribe Ketagalan who settled the type locality of this species (Nakano & Lai 2012). Since the specific name is not a Latin or Latinized word, this specific name is treated as indeclinable.

Orobdella koikei Nakano, 2012
(Figs 12C, 18, 19)

Orobdella koikei Nakano, 2012c: 14–19, figs. 2–5.

Diagnosis. In life, dorsal surface brown. Somite IV basically uniannulate. Somites VIII–XXV quadrannulate. Somite XXVI basically dorsally triannulate, ventrally biannulate. Pharynx reaching to posterior of XIII to anterior of XIV. Gastropore conspicuous in middle of XIII a1. Gastroporal duct basically tubular, but bulbous at junction with crop, winding and slightly bulbous at junction with gastropore. Male gonopore in middle of XI b6, female gonopore inconspicuous in middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli. Paired epididymides in anterior part of XVI to anterior part of XIX, occupying 9–12 annuli. Ejaculatory ducts nearly straight in position anterior to ovisacs. Atrial cornua muscular, ovate.

Type specimens. Holotype: KUZ Z156 (Fig. 18).

Type locality. Sounkyo, alt. 712 m, 43°43′22″N, 142°56′51″E, Kamikawa, Hokkaido, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from

midlength to point just anterior of caudal sucker (Fig. 18), maximum BL 31.5 mm, BW 3.31 mm (KUZ Z151), minimum BL 24.2 mm, BW 2.76 mm (KUZ Z186). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface brown, ventral surface grayish white (Fig. 12C). Color faded in preservative, without any dark lines.

Somite I completely merged with prostomium (Fig. 19A). Somites II, III uniannulate (Fig. 19A); KUZ Z146 slight dorsal furrow present in III. Somite IV almost always uniannulate (Fig. 19A), KUZ Z158 slight dorsal furrow present; KUZ Z146 biannulate. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 19A, B). Somites VI, VII triannulate, a1 = a2 = a3 (Fig. 19A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 19A–E); KUZ Z157 XXV triannulate, a1 = a2 < a3, a3 with slight dorsal furrow. Somite XXVI almost always dorsally triannulate, a1 = a2 = a3, ventrally biannulate a1 < (a2 + a3) (Fig. 19C, D); KUZ Z146 triannulate, a1 = a2 = a3; last annulus of XXVI (frequently (a2 + a3), or scarcely ever a3) being ventrally last complete annulus (Fig. 19D). Somite XXVII almost always uniannulate (Fig. 19C); KUZ Z151 biannulate; anus behind it with no post-anal annulus (Fig. 19C).

Anterior ganglionic mass often in VI a2 and a3; KUZ Z146, Z151 in VI a2 and a3, and VII a1. Ganglion VII often in a2; KUZ Z146, Z158 in a2 and a3. Ganglia VIII, X, XI in a2 and b5 of each somite (Fig. 19H). Ganglion IX generally in a2 and b5; KUZ Z156 in a2. Ganglion XII generally in a2 and b5 (Fig. 19H); KUZ Z151 a2. Ganglion XIII often in a2 and b5; KUZ Z151 in a2; KUZ Z156 in b5 (Fig. 19H). Ganglion XIV generally in a2; KUZ Z156 in a2 and b5 (Fig. 19H). Ganglion XVI often in a2 (Fig. 19H); KUZ Z151, Z158 in a1 and a2. Ganglion XVII often in a2 (Fig. 19H); KUZ Z158 a1 and a2; KUZ Z151 in a2 and b5. Ganglion XVIII generally in a2 (Fig. 19H); KUZ Z146 in a1 and a2. Ganglion XIX often in a1 and a2; KUZ Z156, Z158 in a2. Ganglion XX often in a2; KUZ Z151 in a1; KUZ Z145 in a1 and a2. Ganglion XXI often in a1 and a2; KUZ Z145, Z158 in a2. Ganglion XXII in a1 and a2. Ganglion XXIII often in a1 and a2; KUZ Z145 in a2; KUZ Z151 in b5. Ganglion XXIV occasionally in a1 and a2; KUZ Z156 in a1; KUZ Z145 in a2; KUZ Z151 in b5. Ganglion XXV variably located; KUZ Z146 in XXIV b6; KUZ Z158 in XXIV b6 and XXV a1; KUZ Z156 in XXV a1; KUZ Z151 in XXV a1 and a2; KUZ Z145 in XXV a2. Ganglion XXVI variably located; KUZ Z146, Z158 in XXV b6; KUZ Z145, Z156 in XXV b6; KUZ Z151 in XXVI a1. Posterior ganglionic mass variably located; KUZ Z146, Z158 in

XXV b6, and XXVI a1 and a2; KUZ Z145, Z156 in XXVI a1 and a2; KUZ Z151 in XXVI a2 and a3.

Eyes almost always in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 19A); KUZ Z145 in one pair, without second and third pairs. Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of a1 of each somite (Fig. 19A, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIII b5/b6–XIV a1 (Fig. 19F). Crop tubular, reaching to XIX a2–XX a1. Gastropore conspicuous, ventral, in middle of XIII a1 (Fig. 19E, G). Gastroporal duct often tubular, but bulbous at junction with crop, winding and slightly bulbous at junction with gastropore (Fig. 19F); KUZ Z151, Z158 bulbous, winding at junction with gastropore; joining with crop in XIII b6–XIV a1 (Fig. 19F). Intestine tubular and acecate, reaching to XXIII a1–XXIV b5. Rectum tubular, thin-walled.

Male gonopore in middle of XI b6 (Fig. 19E). Female gonopore in middle of XIII a1, inconspicuous, located posterior to gastropore (Fig. 19G). Gonopores separated by 1/2 + 4 + 1/2 annuli (Fig. 19E). Testisacs multiple, in XIX a1–XIX a2/b5 to XXIII a2–XXV a1 (Fig. 19H). Paired epididymides in XVI a1/a2–XVII a2 to XIX a1–XIX a2/b5 (Fig. 19H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 to XVI a1/a2–XVII a2; nearly straight in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 19H). Pair of muscular atrial cornua, ovate, in XI b5 and b6 (Fig. 19H–K). Atrium short, muscular, globular, in XI b5 and b6 (Fig. 19H–K). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, often in XIII a2 and b5 (Fig. 19H, L); KUZ Z151 in XIII a1–b5; KUZ Z158 in XIII a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII a2 (Fig. 19H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 19L).

Distribution. Known from mountainous regions in Hokkaido, Japan.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Mr. N. Koike who collected the holotype of this species (Nakano 2012c).

Remarks. In the original description, Nakano (2012c) described that the annulation

of somite XXV of this species as being triannulate. However, somite XXV of this species is basically quadrannulate when the specimens used in Nakano (2012c) were reexamination. His description should therefore be emended as described above.

Orobdella mononoke Nakano, 2012
(Figs 12D, 20, 21)

Orobdella mononoke Nakano, 2012a: 83–85, 87–90, figs. 2–5.

Diagnosis. In life, dorsal surface of somites I–XIII, XXVII and caudal sucker bluish gray, and of somites XIV–XXVI amber. Somite III uniannulate. Somite IV basically uniannulate. Somite VI dorsally quadrannulate, ventrally triannulate. Somite VII quadrannulate. Somites VIII–XXV sexannulate. Somite XXVI quinquannulate. Pharynx reaching to XIV. Gastropore conspicuous in middle of XIII b2. Gastroporal duct basically tubular but bulbous at junction with crop, slightly winding at junction with gastropore. Male gonopore in XI c11/c12, female gonopore inconspicuous in middle of XIII b2, behind gastropore, gonopores separated by 8 + 1/2 annuli. Paired epididymides in middle part of XV to posterior part of XIX, occupying 20–22 annuli. Ejaculatory ducts loosely coiled in position anterior to ovisacs. Pre-atrial loops absent. Atrial cornua muscular, ovate.

Type specimens. Holotype: KUZ Z224 (Fig. 20).

Type locality. Shiratani-unsuikyo, alt. 648 m, 30°22′46″N, 130°34′29″E, Yakushima, Yakushima Island, Kagoshima Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 20), maximum BL 139.3 mm, BW 9.2 mm (KUZ Z224), minimum BL 34.1 mm, BW 2.1 mm (KUZ Z222). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface of somites I–XIII, XXVII and caudal sucker bluish gray, and of somites XIV–XXVI amber, ventral surface grayish white (Fig. 12D). Color faded in preservative, without any dark lines.

Somite I completely merged with prostomium (Fig. 21A). Somites II, III uniannulate; KUZ Z224 slight dorsal furrow present in III (Fig. 21A). Somite IV generally

uniannulate, KUZ Z225 slight dorsal furrow present; KUZ Z224 biannulate (Fig. 21A). Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 21A, B). Somite VI dorsally quadrannulate, b1 = b2 < a2 = a3, ventrally triannulate, a1 = a2 = a3 (Fig. 21A, B). Somite VII quadrannulate, a1 = a2 = b5 = b6 (Fig. 21A, B). Somites VIII–XXV sexannulate, b1 = b2 = a2 = b5 = c11 = c12 (Fig. 21A–E). Somite XXVI quinquannulate, b1 = b2 = a2 = b5 > b6, b1 = b2 = a2 = b5 = b6, or b1 = b2 < a2 > b5 = b6; b6 being ventrally last complete annulus (Fig. 21C, D). Somite XXVII uniannulate, biannulate, or triannulate; anus behind it with no post-anal annulus (Fig. 21C).

Anterior ganglionic mass frequently in VI a2 and a3; KUZ Z224 in VI a1 and a2. Ganglion VII frequently in a1 and a2; KUZ Z224 in a1. Ganglia VIII–XII, XIV, XV, XXII, XXIII in a2 of each somite (Fig. 21H). Ganglion XIII frequently in a2; KUZ Z224 in a2 and b5 (Fig. 21H). Ganglia XVI, XX, of each somite, frequently in b2 and a2; KUZ Z224 in a2 (Fig. 21H). Ganglia XVII, XIX, XXI, of each somite, frequently in a2 (Fig. 21H); KUZ Z223 in b2 and a2. Ganglion XVIII frequently in b2 and a2 (Fig. 21H); KUZ Z221 in a2. Ganglion XXIV in b2 and a2. Ganglion XXV frequently in b2; KUZ Z224 in b2 and a2. Ganglion XXVI located variably; KUZ Z223 in XXV c12 and XXVI b1; KUZ Z221 in XXVI b1; KUZ Z224 in XXVI b2. Posterior ganglionic mass located variably; KUZ Z223 in XXVI a2 and b5; KUZ Z221 in XXVI a2–b6; KUZ Z224 in XXVI b5 and b6.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 21A). Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of b2 of each somite (Fig. 21B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV b5/c11–XIV/XV (Fig. 21F). Crop tubular, reaching to XXI b1–a2. Gastropore conspicuous, ventral, slightly anterior to middle of XIII b2 (Fig. 21E, G). Gastroporal duct frequently tubular, but slightly bulbous at junction with crop, winding and slightly bulbous at junction with gastropore (Fig. 21F); KUZ Z223 simple tubular; joining with crop in XIV b5–c11 (Fig. 21F). Intestine tubular and acecate, reaching to XXIV b1–b5. Rectum tubular, thin-walled.

Male gonopore in furrow of XI c11/c12 (Fig. 21E). Female gonopore slightly anterior to middle of XIII b2, inconspicuous, located posterior to gastropore (Fig. 21G). Gonopores separated by 8 + 1/2 annuli (Fig. 21E). Testisacs multiple, in XIX b1–c11 to

XXIV c11–XXV b5 (Fig. 21H). Paired epididymides in XV a2–XVI b2 to XVIII b5–XIX b5 (Fig. 21H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 to XV a2–XVI b2; frequently loosely coiled in position anterior to ovisacs; KUZ Z223 straight in position anterior to ovisacs; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 21H); KUZ Z223 with pre-atrial loop reaching to middle of XI b5. Pair of muscular atrial cornua, frequently ovate, in XI b5 and c11 (Fig. 21H–K); KUZ Z223 fusiform, in XI c11. Atrium short, muscular, globular, in XI c11 and c12 (Fig. 21H–K). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, frequently in XIII a2 and b5 (Fig. 21H, L); KUZ Z223 in XIII a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII b2/a2 (Fig. 21H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 21L).

Distribution. Known from the mountainous regions in Yakushima Island in the Ryukyu Islands, Japan.

Etymology. The specific name is a Japanese word derived from the Japanese animation movie title ‘Mononoke-hime (Princess Mononoke)’, referring that the type locality of this species is the origin of an epic forest in the movie (Nakano 2012a). Since the specific name is not a Latin or Latinized word, this name is treated as indeclinable.

Remarks. The specimen KUZ Z223 possesses pre-atrial loops in its male genital organs. However, on the basis of its possession of undeveloped atrial cornua and ovisacs, this leech was deemed to be an immature individual. Therefore, the pre-atrial loop is considered to be a characteristic only of an immature *Orobdella mononoke*, because mature specimens of this species do not bear such loops.

Orobdella octonaria Oka, 1895
(Figs 22, 23A–D, 24)

Orobdella octonaria Oka, 1895: 286–288, pl. 28, figs. 11–14, pl. 29, fig. C; Oka (1910a): 19; Oka (1910b): 178; Soós (1966): 397; Lukin (1976): 466, 467; Sawyer (1986): 680, 747; Nakano (2012b): 228–232, figs. 2–7.
Kumabdella octonaria: Richardson (1971): 591–599, figs. 1–3.

Diagnosis. Somites IX–XXVI octannulate. Pharynx reaching to posterior part of XIV to XIV/XV. Gastropore conspicuous in middle of XIII b2. Gastroporal duct tubular, bulbous at junction with crop, winding at junction with gastropore. Male gonopore in middle of XI c11, female gonopore inconspicuous, in middle of XIII b2, behind gastropore, gonopores separated by 1/2 + 10 + 1/2 annuli. Paired epididymides in anterior part of XVI to posterior part of XIX, occupying 15–23 annuli. Ejaculatory ducts coiled in position anterior to ovisacs. Atrial cornua muscular, ovate.

Type specimens. Lectotype: NSMT-An 415 (designated by Nakano (2012b)).

Type locality. Mt. Yusakayama (coordinates of the crest: 35°13′41″N, 139°04′46″E), Hakone, Kanagawa Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 22), maximum BL 235.6 mm (KUZ Z545), BW 15.6 mm (KUZ Z552), minimum BL 32.2 mm, BW 2.1 mm (KUZ Z534). Caudal sucker ventral, oval, its greater diameter smaller than BW. Coloration variable; in life, dorsal surface navy blue, dorso-lateral surface of posterior region anterior to caudal sucker slightly yellowish (Fig. 23A), ventral surface bluish white, in preservative, color faded, without any dark lines; in life, dorsal surface bluish gray, dorso-lateral surface in middle to posterior parts of body yellowish, amber, or orange (Fig. 23B), ventral surface bluish white, in preservative, color faded without any dark lines; in life, dorsal surface orange, or orange, but black in somites I–V, and black line reaching to XIV b1 (Fig. 23C), ventral surface yellowish white, or bluish gray, in preservative, color faded, without any dark lines, or with one dorsal dark line in IV–XIV b1; in life, dorsal surface discontinuous black, dorso-lateral surface yellow, amber, or orange (Fig. 23D), ventral surface whitish yellow, in preservative, color faded, but dorsal black pattern remaining; or in life, dorsal surface brown, ventral surface reddish white, or dorsal surface, gray in anterior part of body, and brown in middle part to caudal sucker, ventral surface, gray in anterior part of body, and reddish white in middle part to caudal sucker, in preservative, color faded, without any dark lines.

Somite I completely merged with prostomium (Fig. 24A). Somite II uniannulate. Somites III, IV biannulate (Fig. 24A); KUZ Z543 uniannulate with one slight dorsal furrow. Somite V biannulate, (a1 + a2) > a3 (Fig. 24A, B); KUZ Z545 slight dorsal

furrow present in (a1 + a2); a3 of V forming posterior margin of oral sucker (Fig. 24A, B). Somite VI almost always dorsally sexannulate, b1 = b2 = b3 = b4 = b5 = b6, or b1 = b2 = b3 = b4 = b5 < b6, ventrally quadrannulate, a1 = a2 > b5 = b6, or a1 = a2 > b5 < b6 (Fig. 24A, B); KUZ Z182, Z534 dorsally quadrannulate b1 = b2 = b3 = b4 < a3, ventrally triannulate, a1 = a2 = a3. Somite VII generally dorsally sexannulate, b1 = b2 = b3 = b4 = b5 = b6, or b1 = b2 < b3 = b4 = b5 = b6, ventrally quinquannulate a1 > b3 = b4 = b5 = b6 (Fig. 24A, B); NSMT-An 415, KUZ Z534, Z539 quinquannulate a1 > b3 = b4 = b5 = b6; KUZ Z177 dorsally sexannulate, b1 = b2 = b3 = b4 = b5 = b6, ventrally quadrannulate, a1 = a2 > b5 = b6. Somite VIII dorsally octannulate, b1 = b2 = b3 = b4 < c9 = c10 = c11 = c12, or b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12, ventrally sexannulate a1 = a2 > c9 = c10 = c11 = c12; or rarely octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12; NSMT-An 415, KUZ Z181 dorsally octannulate b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12, ventrally septannulate a1 = b3 = b4 = c9 = c10 = c11 = c12 (Fig. 24A, B); KUZ Z182 septannulate, a1 > b3 = b4 = c9 = c10 = c11 = c12; KUZ Z534 sexannulate, a1 > a2 = c9 = c10 = c11 = c12. Somites IX–XXVI almost always octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12 (Fig. 24C–E); KUZ Z177 IX dorsally octannulate, b1 = b2 = b3 = b4 < c9 = c10 = c11 = c12, ventrally sexannulate a1 = a2 > c9 = c10 = c11 = c12; KUZ Z182 XXVI sexannulate b1 = b2 = b3 = b4 = b5 = b6; c9 of X and c10 of XIII respectively being first and last annuli of clitellum (KUZ Z579). Somite XXVII often triannulate, often slight furrow present in first annulus of XXVII (Fig. 24C); or rarely quadrannulate; KUZ Z586, Z545 dorsally triannulate, ventrally quadrannulate; KUZ Z182, Z546 biannulate; KUZ Z553 quinquannulate; anus behind it with no post-anal annulus (Fig. 24C); generally first annulus of XXVII, or rarely XXVI c12, KUZ Z553, Z570 second annulus of XXVII, KUZ Z182 XXVI b6, being ventrally last complete annulus (Fig. 24D).

Anterior ganglionic mass in VI a2 and b5; KUZ Z537, Z547 in VI a1–b5; KUZ Z551, Z552 in VI b5 and b6; NSMT-An 415 VI a1; KUZ Z570 in VI a1 and a2; KUZ Z554 in VI a2–b6. Ganglion VII generally in a1; KUZ Z570 in VI b6 and VII a1; KUZ Z554 in a1 and b3; KUZ Z551 in b3. Ganglion VIII occasionally in b3 and b4; or rarely in a2; KUZ Z552, Z586 in b3; KUZ Z177 in a1 and a2; KUZ Z570 in b2 and b3; KUZ Z226 in b4. Ganglion IX generally in b3 and b4; KUZ Z177 in a2; KUZ Z570 in b2 and b3; KUZ Z535 in b3. Ganglion X generally in b3 and b4; KUZ Z535, Z552 in b3; KUZ Z554 in b4. Ganglion XI often in b4; occasionally in b3 and b4 (Fig. 24H). Ganglion

XII often in b3 and b4; KUZ Z537, Z540, Z551 in b4; NSMT-An 415, KUZ Z552 in b3 (Fig. 24H). Ganglion XIII often in b4; NSMT-An 415, KUZ Z177, Z570 in b4 and c9 (Fig. 24H); KUZ Z535, Z552 in b3 and b4. Ganglion XIV often in b3 and b4; KUZ Z177, Z540, Z551 in b4; KUZ Z554 in b2 and b3; NSMT-An 415 in b3 (Fig. 24H); KUZ Z570 in c9 and c10. Ganglion XV generally in b3 and b4; NSMT-An 415, KUZ Z177, Z535 in b3 (Fig. 24H). Ganglion XVI in b3; or in b3 and b4 (Fig. 24H). Ganglion XVII often in b3 and b4 (Fig. 24H); or occasionally in b3. Ganglion XVIII often in b3; or occasionally in b3 and b4 (Fig. 24H). Ganglion XIX often in b3; or rarely in b3 and b4; KUZ Z570 in b2 and b3. Ganglion XX in b3 and b4; or rarely in b3; KUZ Z551, Z570 in b2 and b3. Ganglia XXI, XXII, of each somite, often in b3; or occasionally in b3 and b4. Ganglion XXIII generally in b3 and b4; KUZ Z177, Z535 in b3. Ganglion XXIV in b3 and b4; or occasionally in b3; KUZ Z535 in b2 and b3. Ganglion XXV often in b3; KUZ Z177, Z551, Z586 in b2 and b3; KUZ Z535, Z570 in b2. Ganglion XXVI often in b2; KUZ Z181, Z586 in b1 and b2; KUZ Z547, Z579 in b3; NSMT-An 415 in b4; KUZ Z570 in XXV c12 and XXVI b1. Posterior ganglionic mass rarely in XXVI c10–c12; or rarely in XXVI c11, c12 and first annulus of XXVII; KUZ Z540, Z552 in XXVI c10–c12 and first annulus of XXVII; KUZ Z181, Z554 in XXVI c9–c11; KUZ Z551 in XXVI b4–c11; NSMT-An 415 in XXVII.

Eyes generally in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 24A); KUZ Z226 additional one eye dorso-left on VI b2; KUZ Z552, Z553 in one pair,without second and third pairs; NSMT-An 415, KUZ Z570 undetectable. Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of frequently VIII a1 (Fig. 24B); or rarely VIII b2; b2 of each somite in IX–XXIV (Fig. 24E); KUZ Z177 VIII a1, and b2 of each somite in X–XXIV. Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV b1/b2–XIV/XV (Fig. 24F). Crop tubular, reaching to XXI b4–XXII b3. Gastropore conspicuous, ventral, in middle of XIII b2 (Fig. 24E, G). Gastroporal duct tubular, bulbous at junction with crop, slightly winding at junction with gastropore (Fig. 24F); or occasionally bulbous, slightly winding at junction with gastropore; KUZ Z535, Z586 tubular, but winding and slightly bulbous at junction with gastropore; joining with crop in XIV b1–XV b2 (Fig. 24F); KUZ Z586 in XIII c11. Intestine tubular and acecate, reaching to XXIV b4/c9–XXV c9.

Rectum tubular, thin-walled.

Male gonopore often slightly posterior to middle, of XI c11; or occasionally in middle of XI c11 (Fig. 24E). Female gonopore in middle of XIII b2, inconspicuous, located posterior to gastropore (Fig. 24G). Gonopores separated by 1/2 + 10 + 1/2 annuli (Fig. 24E). Testisacs multiple, in XVIII c10–XIX c10 to XXIII c11–XXVI b2. Paired epididymides in XVI b2/b3–XVII b4/c9 to XVIII c10–XIX c9/c10 (Fig. 24H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b4–c9 to XVI b2/b3–XVII b4/c9; coiled, or loosely coiled in position anterior to ovisacs; KUZ Z537, Z554 Z586 straight; coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua (Fig. 24H), with pre-atrial loop reaching to middle of XI b4 to anterior of XI c9; NSMT-An 415 without pre-atrial loop (Fig. 24H). Pair of muscular atrial cornua, frequently ovate; NSMT-An 415, KUZ Z586 fusiform (Fig. 24H–K); KUZ Z226 conical; KUZ Z535 undeveloped; in XI c9–c11; or occasionally in XI c10 and c11 (Fig. 24H); KUZ Z535 in c11. Atrium short, muscular, globular, almost always in XI c11 and c12 (Fig. 24H–K); KUZ Z535 in XI c11. Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, frequently in XIII b3 and b4 (Fig. 24H, L); KUZ Z537, Z579 in XIII b3–c9; KUZ Z181, Z547 in XIII b4. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct generally in XIII b3 (Fig. 24H, L); KUZ Z552, Z554, Z579 in XIII b2/b3. Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 24L).

Distribution. Known from mountainous regions in the central part of Honshu, Japan.

Etymology. The specific name is a feminine Latin adjective (octonary), referring that the mid-body somites of this species are octannulate.

Remarks. The coloration of this species was largely variable. A mature specimen from the type locality possessed a navy blue dorsal surface and a slightly yellowish dorso-lateral surface from the posterior region anterior to the caudal sucker (Fig. 23A). The dorsal surfaces of specimens from the Kii Peninsular were orange, or orange with black somites I–V and a black line reaching to XIV b1 (Fig. 23C). Specimens from other localities had a discontinuous black dorsal surface, and their dorso-lateral surface was yellow, amber, or orange (Fig. 23D). In the other materials, their dorsal surfaces were bluish gray, and the dorso-lateral surfaces were yellowish, amber, or orange in the middle to posterior parts of the body (Fig. 23B). Juvenile specimens possessed brown,

brown gray, or gray dorsal surfaces. The holotype of *O. octonaria* does not have pre-atrial loops. However, the other individuals possess the loops. One specimen, in which the atrial cornua are undeveloped, was demmed to be a juvenile leech due to its possession of undeveloped male genital organs (*e.g.* undetectable testisacs).

Nakano (2012b) emended the description of this species in Richardson (1971).

Orobdella shimadae Nakano, 2011
(Figs 23E, 25, 26)

Orobdella shimadae Nakano, 2011a: 10, 11, figs. 8, 9.

Diagnosis. Somite III uniannulate. Somite IV basically biannulate. Somite VI triannulate. Somite VII triannulate. Somite VIII basically quinquannulate. Somites IX–XXV sexannulate. Somite XXVI basically quinquannulate. Pharynx reaching to XVI. Gastropore absent. Gastroporal duct rudimentary, thin-walled, tubular, but slightly bulbous at female gonopore, not joining with crop, reaching to XV. Male gonopore basically in XI b5/c11, or in anterior margin of XI c11, female gonopore in XIII b1/b2, or in anterior margin of XIII b2, gonopores separated by 9 annuli. Epididymides absent. Sperm ducts coiled in position anterior to ovisacs with pre-atrial loop extending to middle to posterior of XI a2. Atrial cornua absent.

Type specimens. Holotype: KUZ Z128 (Fig. 25).

Type locality. Along the Okuyona Forest Road, alt. 100 m, 26°49.0´N, 128°16.5´E, Kunigami, Okinawajima Island, Okinawa Prefecture, Japan.

Description. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 25), maximum BL 100.9 mm, BW 6.3 mm (KUZ Z128), minimum BL 20.3 mm, BW 1.4 mm (KUZ Z561). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface blackish green, or grayish yellow, dorso-lateral surface yellow, or yellowish white, ventral surface yellow or yellowish white (Fig. 23E). Color faded in preservative, without any dark lines; KUZ Z560, Z563 with dorsal dark line from VII a1–IX a2 to XXVI b6–XXVII.

Somite I completely merged with prostomium (Fig. 26A). Somites II, III uniannulate

(Fig. 26A). Somite IV almost always biannulate (Fig. 26A); KUZ Z561 uniannulate. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 26A, B). Somite VI triannulate, a1 = a2 = a3 (Fig. 26A, B). Somite VII triannulate, a1 = a2 < a3 (Fig. 26A, B), in a3, KUZ Z125 slight dorsal furrow present, KUZ Z558 slight furrow present. Somite VIII almost always quinquannulate, a1 > a2 = b5 > c11 = c12 (Fig. 26A, B), in a1, KUZ Z562 slight dorsal furrow present, KUZ Z558 slight furrow present; KUZ Z561, Z564 quadrannulate, a1 > a2 = b5 < b6, in b6, KUZ Z564 slight dorsal furrow present, KUZ Z561 slight furrow present. Somites IX–XXV sexannulate, anterior part of somites, b1 = b2 < a2 = b5 > c11 = c12 (Fig. 26C–E); middle and posterior part of somites, b1 = b2 = a2 = b5 = c11 = c12; KUZ Z564 IX quinquannulate, a1 > a2 = b5 > c11 = c12, slight dorsal furrow present in a1; b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z125–Z128) (Fig. 26E). Somite XXVI frequently quinquannulate, sexannulate, annulation hardly decidable, possibly a1 = a2 = b5 > c11 = c12, or b1 = b2 < a2 > b5 = b6, KUZ Z566 slight dorsal furrow present in b6; KUZ Z560, Z562, Z563 dorsally sexannulate, ventrally quinquannulate; KUZ Z128, Z139 sexannulate b1 = b2 = a2 = b5 > c11 = c12 (Fig. 26C, D); last annulus of XXVI (b6 or c12) being ventrally last complete annulus (Fig. 26D). Somite XXVII biannulate (Fig. 26C); rarely uniannulate; or rarely triannulate; anus behind it with no post-anal annulus (Fig. 26C).

Anterior ganglionic mass in VI a2 and a3. Ganglia VII, X, XII, XVII in a2 of each somite. Ganglion VIII frequently in a2; KUZ Z127, Z131, Z560 in a2 and b5. Ganglia IX, XI, XVI, XVIII, of each somite, almost always in a2 (Fig. 26H); KUZ Z127 ganglion IX in a2 and b5; KUZ Z558 ganglion XI in b2 and a2; KUZ Z563 ganglion XVI in b2 and a2; KUZ Z566 ganglion XVIII in b2 and a2. Ganglion XIII frequently in a2; or occasionally in a2 and b5 (Fig. 26H). Ganglia XIV, XV of each somite, generally in a2 (Fig. 26H); KUZ Z130 in a2 and b5; KUZ Z560 ganglion XIV in a2 and b5; KUZ Z563 ganglion XV in a2 and b5. Ganglion XIX generally in a2; KUZ Z563, Z566 in b2 and a2. Ganglion XX frequently in a2; KUZ Z131, Z558, Z560 in b2 and a2. Ganglion XXI almost always in b2 and a2; KUZ Z566 in a2. Ganglion XXII frequently in a2; KUZ Z127, Z131, Z138 in b2 and a2. Ganglion XXIII often in a2; or occasionally in b2 and a2. Ganglion XXIV frequently in b2 and a2; KUZ Z127, Z128, Z563 in a2. Ganglion XXV located variably; KUZ Z127, Z131, Z138 in b1 and b2; KUZ Z130, Z560, Z563 in b2 and a2; KUZ Z128 in a2; KUZ Z566 in b1; KUZ Z558 in b2.

Ganglion XXVI frequently in first annulus of XXVI 8a1 or b1); KUZ Z131. Z560 in XXV c12 and XXVI b1; KUZ Z566 in XXV c12; KUZ Z130 in a2. Posterior ganglionic mass located variably; KUZ Z131, Z560, Z563 in XXVI a2 to last annulus of XXVI (b6, or c11); KUZ Z128, Z558 in XXVI a2 and b5; KUZ Z127, Z138 in XXVI b5–c12; KUZ Z558 in XXVI a2 and b5; KUZ Z130 in XXVI c11, c12.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 26A). Nephridiopores almost always in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of VIII a1, and b2 of each somite of IX–XXIV (Fig. 26B, E); KUZ Z564, Z565 in 18 pairs in VIII–XXV, KUZ Z565 situated ventrally at posterior margin of VIII a1, and b2 of each somite of IX–XXV, or KUZ Z564 at posterior margin of a1 of each VIII, IX, and b2 of each somite of X–XXV. Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XV/XVI–XVI c11/c12 (Fig. 26F). Crop tubular, reaching to XXII b2–XXII c11/c12. Gastropore absent. Gastroporal duct rudimentary, thin-walled, tubular, but slightly bulbous at female gonopore, not joining with crop, reaching to XV a2–XV b5 (Fig. 26F). Intestine tubular and acecate, reaching to XXIV b5/c11–XXV b2/a2. Rectum tubular, thin-walled.

Male gonopore often in furrow of XI b5/c11; or occasionally in anterior margin of XI c11 (Fig. 26E); KUZ Z563 in middle of XI c11. Female gonopore often in furrow of XIII b1/b2; or occasionally in anterior margin of XIII b2 (Fig. 26E). Gonopores separated by almost always 9 annuli (Fig. 26E); KUZ Z563 1/2 + 8 annuli. Testisacs multiple, in XVI c11–XVIII a2 to XXIV a2–XXV c11/c12. Sperm ducts in XI a2 to XVI c11–XVIII a2; coiled in position anterior to ovisacs; hardly coiled in position posterior to ovisacs; each widening from respective junction with testisacs, narrowing at junction with atrium, with pre-atrial loop extending to middle to posterior of XI a2 (Fig. 26H, I). Epididymides absent. Ejaculatory bulbs absent. Atrial cornua absent. Atrium short, muscular, globular, in XI b5 and c11 (Fig. 26H, I). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, generally in XIII a2 and b5 (Fig. 26H, J); KUZ Z131, Z560 in XIII a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII b2 (Fig. 26H, J). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 26J).

Distribution. Known from mountainous regions in the northern and central parts of Okinawajima Island in the Ryukyu Islands, Japan.

Etymology. The specific name is a noun in the genitive case formed from the Latinized name of Mr. T. Shimada who collected the holotype of this species (Nakano 2011a).

Remarks. One specimen KUZ Z563 from Mt. Katsuudake in the Motobu Peninsula of Okinawajima Island has a male gonopore in the middle part of XI c11 in contrast to a male gonopore that opens in the furrow of XI b5/c11, or the anterior margin of XI c11 in the other specimens of *O. shimadae*. Except for the position of its male gonopore, the specimen from Mt. Katsuudake possesses the diagnostic characteristics of *O. shimadae*. The molecular phylogenies obtained in this study indicated that this specimen was clearly placed within the clade of *O. shimadae* (see below).

Orobdella tsushimensis Nakano, 2011
(Figs 23F, 27, 28)

Orobdella tsushimensis Nakano, 2011b: 41–46, figs. 2–4; Nakano & Seo (2012): 237–239, figs. 2–4.

Diagnosis. Somite IV uniannulate. Somites VIII–XXV quadrannulate. Somite XXVI triannulate. Pharynx reaching to XIV. Gastropore conspicuous in XIII a1/a2. Gastroporal duct basically bulbous, slightly winding at junction with gastropore. Male gonopore in middle of XI b6 (slightly anterior to middle of annulus), female gonopore inconspicuous in furrow of XIII a1/a2, behind gastropore, gonopores separated by 1/2 + 5 annuli. Paired epididymides in posterior part of XVI to posterior part of XIX, occupying 8–10 annuli. Ejaculatory ducts straight in position anterior to ovisacs. Atrial cornua muscular, ovate or conical.

Type specimens. Holotype: KUZ Z134 (Fig. 27).

Type locality. Near the Orobashi Bridge at the Kechi Dam, alt. 69m, 34°15′17″N, 129°17′17″E, Tsushima, Tsushima Island, Nagasaki Prefecture, Japan.

Redescription. Body firm and muscular, elongate, gaining regularly in width in caudal direction in cephalic part, dorso-ventrally depressed, sides nearly parallel from midlength to point just anterior of caudal sucker (Fig. 27), maximum BL 112.5 mm,

BW 9.38 mm (KUZ Z213), minimum BL 40.2 mm, BW 2.84 mm (KUZ Z214). Caudal sucker ventral, oval, its greater diameter smaller than BW. In life, dorsal surface black olive, dorso-lateral surface yellow, or amber, ventral surface whitish yellow (Fig. 23F). Color faded in preservative, frequently with median dorsal black line on posterior part to XXVII; or rarely without any dark lines; KUZ Z215 with one dorsal dark line from VIII a1 to XIII a2.

Somite I completely merged with prostomium (Fig. 28A). Somites II, III uniannulate (Fig. 28A); KUZ Z215 slight dorsal furrow present in III. Somite IV uniannulate (Fig. 28A), rarely slight dorsal furrow present. Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 28A, B). Somites VI–VII triannulate, a1 = a2 = a3 (Fig. 28A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 28A–E); b5 of X and a2 of XIII respectively being first and last annuli of clitellum (KUZ Z213). Somite XXVI triannulate, a1 = a2 = a3, a1 = a2 < a3, or a1 > a2 > a3; generally a3 being ventrally last complete annulus (Fig. 28C, D); KUZ Z136, Z173 a2 being ventrally last complete annulus. Somite XXVII often uniannulate (Fig. 28C); or rarely uniannulate with one slight dorsal furrow; KUZ Z213, Z215 triannulate; anus behind it with no post-anal annulus (Fig. 28C).

Anterior ganglionic mass in VI a2 and a3. Ganglia VII–X, XV–XVII, XIX, XXI in a2 of each somite (Fig. 28H). Ganglion XI frequently in a2 and b5 (Fig. 28H); KUZ Z213, Z215 in a2. Ganglion XII generally in a2 (Fig. 28H); KUZ Z133 in a2 and b5. Ganglion XIII frequently in a2 and b5 (Fig. 28H); KUZ Z135, Z215 in b5. Ganglion XIV generally in a2 (Fig. 28H); KUZ Z213 in a2 and b5. Ganglion XVIII frequently in a2 (Fig. 28H); KUZ Z213, Z215 in a1 and a2. Ganglion XXII frequently in a2; KUZ Z136, Z213 in a1 and a2. Ganglion XXIII frequently in a1 and a2; KUZ Z133, Z136 in a2. Ganglion XXIV in a1 and a2. Ganglion XXV frequently in a1; KUZ Z134, Z215 in a1 and a2. Ganglion XXVI occasionally in a1; KUZ Z136 in XXV b5 and b6; KUZ Z213 in XXV b6; KUZ Z133 in XXV b6 and XXVI a1. Posterior ganglionic mass often in XXVI a2 and a3; KUZ Z136 in XXVI a1 and a2; KUZ Z133 in XXVI a1–a3.

Eyes generally in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorso-laterally on posterior margin of V (a1 + a2) (Fig. 28A); KUZ Z215 in two pairs, lacking first pair; KUZ Z213 undetectable. Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at posterior margin of a1 of each somite (Fig. 28B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV a1/a2–b5 (Fig. 28F). Crop tubular, reaching to XIX b5/b6–XX a1. Gastropore conspicuous, ventral, in furrow of XIII a1/a2 (Fig. 28E, G). Gastroporal duct often bulbous, slightly winding at junction with gastropore (Fig. 28F); KUZ Z133, Z136 tubular, but slightly bulbous at junctions with gastropore and crop, respectively; joining with crop in XIV a1–a2/b5 (Fig. 28F). Intestine tubular and acecate, reaching to XXIII a1–XXIV/XXV. Rectum tubular, thin-walled.

Male gonopore slightly anterior to middle of XI b6 (Fig. 28E). Female gonopore in furrow of XIII a1/a2, inconspicuous, located posterior to gastropore (Fig. 28G). Gonopores separated by 1/2 + 5 annuli (Fig. 28E). Testisacs multiple, in XIX a2–XIX b5/b6 to XXIII a2–XXV b6 (Fig. 28H). Paired epididymides in XVI b5/b6–XVII a2/b5 to XVIII/XIX–XIX b5/b6 (Fig. 28H). Ejaculatory bulbs absent. Ejaculatory ducts in XI b5 to XVI b5/b6–XVII a2/b5; generally straight in position anterior to ovisacs; KUZ Z134 loosely coiled in position anterior to ovisacs; coiled in position to posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning gradually inward toward atrial cornua without pre-atrial loop (Fig. 28H). Pair of muscular atrial cornua, frequently ovate; KUZ Z134, Z136 conical (Fig. 28H–K); in XI b5 and b6 (Fig. 28H). Atrium short, muscular, globular, in XI b5 and b6 (Fig. 28H–K). Penis sheath and penis absent. One pair of ovisacs, thin-walled, globular, generally in XIII a2 and b5 (Fig. 28H, L); KUZ Z213 in a2. Oviducts thin-walled, right or left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a2 (Fig. 28H, L). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 28L).

Distribution. Known from mountainous regions in Tsushima Island, Japan, and Gageodo Island, South Korea.

Etymology. The specific name is a feminine adjective derived from the name of the type locality of this species (Nakano 2011b).

Remarks. The specimens from Tsushima,Island where it is the type locality of this species, has a yellow dorso-lateral surface in contrast to the amber dorso-lateral surface of the specimens from Gageodo Island. Although the morphology of the gastroporal duct and the atrial cornua of this species are variable, this species can be identified on the basis of its possession of 1/2 + 5 annuli between the gonopores.

Phylogenetic relationships and genetic distances

The ML tree with ln L = −23905.90 (Fig. 29) was almost identical to the obtained BI tree (not shown). The following relationships were indicated by the two analyses:

1. Monophyly of the eleven known species of *Orobdella* was well supported (BS = 100%, BPP = 100%). Monophyly of *Orobdella* and *Gastrostomobdella* was also well supported (BS = 73%, BPP = 99%; not shown).
2. *Orobdella* was divided into two subclades: Clade A (BS = 100%, BPP = 100%) consisting of two species from Hokkaido, *O. kawakatsuorum* and *O. koikei*; and Clade B (BS = 91%, BPP = 100%) composed of the other nine species distributed in areas south of Hokkaido.
3. In Clade A, monophyly of *O. kawakatsuorum*, and *O. koikei* was well recovered (*O. kawakatsuorum*: BS = 97%, BPP = 100%; *O. koikei*: BS = 85%, BPP = 99%).
4. Clade B was split into four subclades: Clade B1 (BS = 96%, BPP = 100%) including five species, *O. mononoke*, *O. esulcata*, *O. ketagalan*, *O. dolichopharynx*, and *O. shimadae*; Clade B2 (BS = 98%, BPP = 100%) consisting of two species, *O. ijimai* and *O. octonaria*; Clade B3 (BS = 100%, BPP = 100%) composed of only *O. tsushimensis*; and Clade B4 (BS = 97%, BPP = 100%) including only *O. whitmani*.
5. In Clade B1, *O. mononoke* and *O. esulcata* formed a monophyletic clade, but with low support in the ML tree (BS = 49%, BPP = 96%). Monophyly of *O. mononoke*, and *O. esulcata* was well recovered (*O. mononoke*: BS = 100%, BPP = 100%; *O. esulcata*: BS = 100%. BPP = 100%). *Orobdella ketagalan*, *O. dolichopharynx* and *O. shimadae* formed a monophyletic clade, but was not well supported by the ML analysis (BS = 69%, BPP = 100%). *Orobdella dolichopharynx* and *O. shimadae* formed a monophyletic clade (BS = 90%, BPP = 100%). Monophyly of *O. dolichopharynx*, and *O. shimadae* was well supported (*O. dolichopharynx*: BS = 86%, BPP = 99%; *O. shimadae*: BS = 100%, BPP = 100%).
6. Clades B2–B4 formed a monophyletic clade, but with low support (BS = 36%, BPP = 84%). In Clade B2, monophyly of the sexannulate *O. ijimai*, and the octannulate *O. octonaria* was well recovered (*O. ijimai*: BS = 100%, BPP = 100%; *O. octonaria*: BS = 91%, BPP = 99%).
7. Clades B3–B4 formed a monophyletic clade, but it was not well supported (BS = 46%, BPP = 87%).
8. Clade B4 was divided into three subclades: Clade B4a (BS = 100%, BPP = 100%)

consisting of *O. whitmani* from the Chugoku Region, Honshu, Japan; Clade B4b (BS = 97%, BPP = 100%) composed of *O. whitmani* from the central part of Honshu, including the specimens from the type locality; and Clade B4c (BS = 99%, BPP = 100%) consisting of *O. whitmani* from the Kinki Region, Honshu. Clades B4a and B4b formed a monophyletic clade (BS = 71%, BPP = 97%).

The interspecific variation in COI sequences between *O. koikei* and *O. kawakatsuorum* was between 8.1% and 9.9% (mean = 9.0%). The intraspecific K2P distances of COI sequences in each species were as follows: *O. kawakatsuorum*, range 0.8–5.0% (mean = 3.7%); *O. koikei*, range 4.8–7.8% (mean = 7.2%); *O. mononoke*, 0.2%; *O. esulcata*, range 0.2–6.5 (mean = 4.7%); *O. dolichopharynx*, range 2.8–9.5% (mean = 6.6%); *O. shimadae*, range 0.6–5.5% (mean = 4.2%); *O. ijimai*, range 5.3–8.4% (mean = 6.9%); *O. octonaria*, range 0.2–8.7% (mean = 6.6%); *O. tsushimensis*, between 2.2–5.5% (mean = 4.3%); and *O. whitmani*, between 0.1–10.4% (mean = 7.5%). In *O. dolichopharynx*, the COI K2P distance between the specimen from Tokunoshima Island and those from Amami-oshima Island ranged between 7.9% and 9.5% (mean = 8.5%). The COI K2P distance within each subclade of *O. whitmani* was; range 4.6–5.9% (mean = 5.2%) in Clade B4a; range 2.8–8.3% (mean = 6.2%) in B4b; and in B4c, range 0.1–7.2% (mean = 5.1%). The COI K2P distances among the three subclades of *O. whitmani* were as follows: between B4a and B4b, range 6.9–10.1% (mean = 8.7%); between B4a and B4c, range 6.9–8.7% (mean = 7.7%); and between B4b and B4c, range 7.4–10.4% (mean = 8.8%).

Discussion

Phylogenetic relationships with geographical distribution

In contrast to the molecular phylogenies in the original description of Orobdellidae in Nakano *et al.* (2012), the present phylogenetic analyses suggest that *Orobdella* formed a monophyletic clade with *Gastrostomobdella*. The present phylogenies recovered the monophyly of the genus *Orobdella* well. The geographical distribution of each species with its subclade name is shown in Figure 30.

Two species *O. kawakatsuorum* and *O. koikei*, which are distributed in Hokkaido, Japan, formed a monophyletic clade (Clade A). These two species inhabit locations in Hokkaido sympatrically. Clade A was a sister group of the other *Orobdella* species (Clade B). Clade B included nine *Orobdella* species that inhabit locations south of Hokkaido, and was divided into four subclades (B1–4). However, in the phylogenetic analyses undertaken in this study, the phylogenetic relationships of these subclades were not sufficiently resolved.

Clade B1 consisted of five species from Kyushu and the Ryukyu Islands, Japan and Taiwan. Clade B1 included three *Orobdella* species from the Ryukyu Islands, *viz*., *O. dolichopharynx* from Amami-oshima Island, Kakeromajima Island and Tokunoshima Island, *O. shimadae* from Okinawajima Island, and *O. mononoke* from Yakushima Island. Yakushima Island is located in the northern part of the Ryukyu Islands. Amami-oshima Island, Tokunoshima Island and Okinawajima Island are located in the middle region of the Ryukyu Islands. These three species did not form a monophyletic clade. This result indicates that *Orobdella* species in the Ryukyu Islands have different evolutionary and biogeographical histories as noted by Nakano (2012a). In the present phylogenies, *Orobdella mononoke* from Yakushima Island formed a monophyletic clade with *O. esulcata* from northern Kyushu, with low statistical support in the ML tree (BS = 49%, BPP = 96%). In contrast, the two species *O. dolichopharynx* and *O. shimadae* from the middle region of the Ryukyu Islands formed a monophyletic clade. This clade is a sister group of *O. ketagalan* from Taipei, Taiwan. However, this relationship was not well supported in the ML tree (BS = 69%, BPP = 100%).

Clade B2 included *O. ijimai* and *O. octonaria*. This relationship was also recovered in previous phylogenetic studies (Nakano 2012a, c; Nakano & Lai 2012; Nakano & Seo 2012; Nakano *et al.* 2012). *Orobdella ijimai* is distributed in the northern Kanto Region in Honshu. The distribution range of this species slightly overlaps that of the leeches of

O. *whitmani* that belong to Clade B4b. *Orobdella octonaria* was collected from a wide region of central Honshu. *Orobdella octonaria* is distributed sympatrically with *Orobdella whitmani*: being sympatric in the Tokai Region with *O*. *whitmani* that belongs to Clade B4b; and in the Kii Peninsula with *O*. *whitmani* that belongs to Clade B4c.

Clade B3 consisted only of quadrannulate *O*. *tsushimensis* from Tsushima Island, Japan, and Gegeodo Island, South Korea. *Orobdella tsushimensis* from Tsushima Island formed a monophyletic clade. In the present phylogenies, Clade B3 formed a monophyletic clade with Clade B4, which consisted of *O*. *whitmani*, with low support (BS = 46%, BPP = 87%). Previous molecular phylogenetic studies showed that this species was clearly included into a clade that consisted of the *Orobdella* species distributed in the areas south of Hokkaido (Nakano 2012a, c; Nakano & Lai 2012; Nakano & Seo 2012; Nakano *et al.* 2012). However, the phylogenetic position of *O*. *tsushimensis* remains uncertain. This species was a sister taxon of the other *Orobdella* species (fig.6 in Nakano (2012a), and fig. 5 in Nakano & Lai (2012)). Conversely, *O*. *tsushimensis* was related to the clade ((*O*. *octonaria* + *O*. *ijimai*) + *O*. *whitmani*) (fig. 5 in Nakano & Seo (2012)).

Clade B4 consisted of populations of *O*. *whitmani*. This clade was divided into three subclades, B4a–c. Clade B4a consisted of specimens from the Chugoku Region in Honshu, and Shikoku. Clade B4b included the leeches mainly from the Chubu Region and Tokai Region in Honshu, and also included the topotype of *O*. *whitmani*. Clade B4c consisted of specimens from the Kinki Region in Honshu. From the geographical distribution of each subclade, it would be expected that Clade B4b would be closely related to Clade B4c because the distribution of Clade B4b is adjacent to that of Clade B4c. However, Clade B4b formed a monophyletic clade with Clade B4a (BS = 71%, BPP = 97%). The present phylogenies suggest that Clade B4c may have a unique biogeographical history in contrast to Clades B4a and B4b.

Morphological characters related to phylogenetic relationships

Previous molecular phylogenetic studies have revealed that sexannulation has evolved in parallel in this genus (Nakano 2012a, c; Nakano & Lai 2012; Nakano & Seo 2012; Nakano *et al.* 2012). The present phylogenies also indicate this morphological evolution. Three sexannulate species, *O*. *mononoke*, *O*. *shimadae*, and *O*. *dolichopharynx*, belong

to Clade B1. The remaining sexannulate species, *O. ijimai*, was included in Clade B2. The octannulate *O. octonaria* was a sister group to the sexannulate *O. ijimai*. These phylogenetic relationships suggest that octannulation may have evolved from a sexannulate species, and not from a quadrannulate species. In the original description of *Kumabdella*, Richardson (1971) mentioned that the genus *Orobdella* should only include the quadrannulate *O. whitmani*, and a new genus should be established for the sexannulate *O. ijimai*. If his classification is followed, several new genera should be established for the quadrannulate, and sexannulate species according to the present phylogenies. Therefore, the present phylogenetic analyses also suggest that Richardson's classification of *Orobdella* should be reconsidered. Only sexannulate and octannulate species of *Orobdella* are reported in the Japanese Archipelago. Gilyarov *et al.* (1969) reported an undescribed quadrannulate species (*O. whitmani* in their study) from Primorsky Krai, Russia. From South Korea, only the quadrannulate *O. tsushimensis* was recorded (Nakano & Seo 2012). In addition, *O. ketagalan* from Taiwan is also a quadrannulate species. Therefore, the annulation in *Orobdella* is considered to have diversified only in the species inhabiting the Japanese Archipelago.

The species that possess a gastroporal duct, which is tubular, and not bulbous at the junction with a crop, belonged to two clades: *O. kawakatsuorum* belonged to Clade A; and two species, *O. esulcata*, *O. ketagalan*, belonged to Clade B1. Therefore, a tubular gastroporal duct is not related to the phylogenetic relationships of *Orobdella*. Two species, *O. dolichopharynx* and *O. shimadae*, have a rudimentary gastroporal duct. Their duct do not open to either the gastropore or the crop. According to the present phylogenies, the clade of these two species was a sister group to *O. ketagalan*, which possesses a simple tubular gastroporal duct. The duct of *O. ketagalan* is thin-walled and narrow. These results indicate that a rudimentary gastroporal duct has evolved from a simple tubular duct. For a better understanding of the evolutionary history of gastroporal ducts, the function of this duct should be revealed.

The degeneration of epididymides is also considered to have evolved in parallel in *Orobdella*. *Orobdella kawakatsuorum* has very short epididymides, which occupy only 1–2 annuli. *Orobdella ketagalan*, *O. dolichopharynx*, and *O. shimadae* lack epididymides in their male genital organs. The absence of epididymides may therefore be a synapomorphy of these three species.

Genetic distances and cryptic diversity

Nakano (2012c) noted that the COI K2P distance (range 8.1–9.9%, mean = 9.0%) between *O. kawakatsuorum* and *O. koikei* could be used to determine whether *Orobdella* species are distinct species or not, because these two species are distributed sympatrically. In the present study, interspecific variation between these two species was the same as reported in Nakano (2012c) (range 8.1–9.9%, mean = 9.0%). As described above, *O. dolichopharynx* from Tokunoshima Island in the Ryukyu Islands possesses a male gonopore in the middle of XI c11 in contrast to a male gonopore in the furrow of XI c11/c12, or in the anterior margin of XI c12 of the specimens of *O. dolichopharynx* from Amami-oshima Island and Kakeromajima Island in the Ryukyu Islands. The COI K2P distance between *O. dolichopharynx* from Tokunoshima Island and that from the other two islands was 7.9–9.5% (mean = 8.5%). This large value suggests that *Orobdella* specimens from Tokunoshima Island could be treated as a distinct species, or a subspecies of *O. dolichopharynx*.

As mentioned above, Clade B4, which consisted of *O. whitmani*, was divided into three subclades (B4a–c) in the phylogenies presented here. The COI K2P distances among these subclades were also determined to be large: between B4a and B4b, range 6.9–10.1% (mean = 8.7%); between B4a and B4c, range 6.9–8.7% (mean = 7.7%); and between B4b and B4c, range 7.4–10.4% (mean = 8.8%). These values also indicate that each of these subclades could be considered a distinct species. Clade B4b included a specimen from the type locality of *O. whitmani*. Thus, this subclade was considered a named species, *O. whitmani*. The remaining two subclades, B4a, and B4c could be considered to be undescribed species, on the basis of the molecular analyses undertaken here. The specimens of Clade B4c have 19 pairs of nephridiopores in contrast to the 17 pairs of nephridiopores in the specimens of Clades B4a and B4b. The specimens that belong to Clade B4a could be distinguished from specimens of Clade B4b due to their possession of short epididymides, which occupy 6–7 annuli in XV/XVI–XVI a1/a2 to XVII b5–XVII b5/b6 (8–11 annuli in XV a2/b5–16a2 to XVII b5/b6–XVIII/XIX in specimens of Clade B4b).

However, the number of specimens of each subclade is still too limited to determine whether each of these cryptic clades is a distinctive undescribed species or not, and to further describe them as a new species. In the present study, only one *Orobdella* specimen from Tokunoshima Island was examined. The geographical range of the *O.*

whitmani specimens collected did not fully cover Honshu. Therefore, in the present study, these subclades were not considered distinctive species as described above.

Comparisons and key

Although the mid-body somite annulation does not reflect the phylogenetic relationships of *Orobdella*, three characteristics of annulation, *viz*., quadrannulate, sexannulate, or octannulate, are important for determining the species identity of an individual of *Orobdella*. First, only *O. octonaria* is known as a species, in which mid-body somites are octannulate. Six quadrannulate species can be distinguished from each other by the following combination of characters: annulation of IV; annulation of XXIV; number of annuli between male and female gonopores; morphology of gastroporal duct; length of epididymides; with or without paired sperm duct bulbs; morphology of ejaculatory ducts in position anterior to ovisacs; and morphology of atrial cornua (Table 3). Four sexannulate species differ from each other in the characteristics of the following combination of characters: annulation of III; annulation of IV; annulation of VI; annulation of VII; annulation of VIII; annulation of XXVI; number of annuli between gonopores; length of pharynx; morphology of gastroporal duct; length of epididymides; morphology of epididymides in position anterior to ovisacs; with or without pre-atrial loop; and morphology of atrial cornua (Table 4).

The identification key to the eleven known *Orobdella* species is as follows:

1 Mid-body somites more than quadrannulate.....2
- Mid-body somites quadrannulate.....6
2 Mid-body somites sexannulate.....3
- Mid-body somites octannulate.....***Orobdella octonaria*** Oka, 1895
3 Pharynx reaching to XIV.....4
- Pharynx reaching to XVI.....5
4 Gonopores separated by 1/2 + 7 + 1/2 annuli.....***Orobdella ijimai*** Oka, 1895
- Gonopores separated by 8 + 1/2 annuli. In life, dorsal surface bicolor, grayish purple and amber.....***Orobdella mononoke*** Nakano, 2012
5 Gonopores separated by 8 annuli.....***Orobdella dolichopharynx*** Nakano, 2011
- Gonopores separated by 9 annuli.....***Orobdella shimadae*** Nakano, 2011
6 Gonopores separated by 1/2 + 4 + 1/2 annuli.....8
- Gonopores separated not by 1/2 + 4 + 1/2 annuli.....7

7 Gonopores separated by 6 annuli.....***Orobdella kawakatsuorum*** Richardson, 1975

- Gonopores separated by 1/2 + 5 annuli.....***Orobdella tsushimensis*** Nakano, 2011

8 Epididymides present.....9

- Epididymides absent, small paired sperm duct bulbs in XV.....
  .....***Orobdella ketagalan*** Nakano & Lai, 2012

9 Gastroporal duct bulbous at junction with crop.....10

- Gastroporal duct tubular, but bulbous only at junction with gastropore.....
  .....***Orobdella esulcata*** Nakano, 2010

10 Somite XXV triannulate.....***Orobdella koikei*** Nakano, 2012

- Somite XXV quadrannulate.....***Orobdella whitmani*** Oka, 1895

Conclusion

Redescription of eleven known *Orobdella* species, and their phylogenetic relationships were presented. The molecular phylogenies and COI K2P distances presented here suggest that several cryptic species still exist within the known species. In addition to these, several undescribed species of *Orobdella* are distributed in Japan (Nakano, unpublished data), and Taiwan (Nakano & Lai, unpublished data). The quadrannulate *Orobdella* species are also distributed in the Korean Peninsula (Nakano & Seo, unpublished data). The species identity of *Orobdella* leeches from the Russian Far East is still unresolved. The existing faunal surveys and taxonomic studies of *Orobdella* are still insufficient to completely reveal the species diversity of this genus. The phylogenetic analyses undertaken here could not recover more detailed phylogenetic relationships of *Orobdella*. Especially in Clade B, the phylogenies presented here failed to reveal the relationships of each subclade. For the purpose of the ancestral state reconstruction of each character in *Orobdella*, more robust trees for this genus based on either more DNA markers or specimens should be obtained. Robust phylogenies are also crucial to better understand the biogeographical history of this genus. Although this study provided provisional taxonomic information for known *Orobdella* species, further systematic studies should be conducted based on this systematic revision.

Systematic studies on the terrestrial macrophagous leeches are vastly limited. In Asia, two such genera, *Orobdella* and *Gastrostomobdella*, have been reported. Although these two genera clearly belong to the macrophagous suborder Erpobdelliformes, their phylogenetic position within Erpobdelliformes still remains uncertain. In addition, they have a unique mid-gut organ (the gastroporal duct). Their gastroporal ducts are highly muscularized, and are a noteworthy organ within the subclass Hirudinida. To understand their evolutionary history, it is crucial to revel the function of their gastroporal duct. The species diversity of these terrestrial macrophagous leeches in Asia is still unclear. Further faunistic, morphological, and systematic studies should be undertaken to reveal the diversity and evolutionary history of the Asian macrophagous leeches to further our understanding of the full evolutionary history of Hirudinida.

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Tables

Table 1. Eleven known species of the genus *Orobdella* with the information on annulation, and their country or region of distribution.

Taxon	Annulation	Distribution
Type species		
Orobdella whitmani Oka, 1895	quadr-	Honshu, and Shikoku, JPN
Additional species		
Orobdella dolichopharynx Nakano, 2011	sex-	Amami-oshima Is., RYK, JPN
Orobdella esulcata Nakano, 2010	quadr-	Kyushu, JPN
Orobdella ijimai Oka, 1895	sex-	Honshu, JPN
Orobdella kawakatsuorum Richardson, 1975	quadr-	Hokkaido, JPN
Orobdella ketagalan Nakano & Lai, 2012	quadr-	Taipei, TWN
Orobdella koikei Nakano, 2012	quadr-	Hokkaido, JPN
Orobdella mononoke Nakano, 2012	sex-	Yakushima Is., RYK, JPN
Orobdella octonaria Oka, 1895	oct-	Honshu, JPN
Orobdella shimadae Nakano, 2011	sex-	Okinawajima Is., RYK, JPN
Orobdella tsushimensis Nakano, 2011	quadr-	Tsushima Is., JPN, and Gageodo Is., KOR

Abbreviations: KOR, Korea; JPN, Japan; TWN, Taiwan; RYK, Ryukyu Islands.

Table 2. Samples used for the phylogenetic analyses, with the information on voucher, and locality.

Voucher	Locality (locality no.)	COI	$tRNA^{Cys}$–16S
Orobdella whitmani			
KUZ Z40	Kyoto, Kyoto, JPN (44)	This study	This study
KUZ Z45 (topotype)	Gifu, Gifu, JPN (32)	AB679668[a]	AB679669[a]
KUZ Z144	Nagano, Nagano, JPN (26)	This study	This study
KUZ Z160	Sumoto, Gifu, JPN (34)	This study	This study
KUZ Z163	Sumoto, Gifu, JPN (33)	This study	This study
KUZ Z164	Maibara, Shiga, JPN (36)	This study	This study
KUZ Z191	Nagahama, Shiga, JPN (37)	AB679670[a]	AB679671[a]
KUZ Z525	Kyoto, Kyoto, JPN (43)	This study	This study
KUZ Z528	Otsu, Shiga, JPN (41)	This study	This study
KUZ Z529	Takayama, Gifu, JPN (31)	This study	This study
KUZ Z532	Kyoto, Kyoto, JPN (44)	This study	This study
KUZ Z533	Kyoto, Kyoto, JPN (43)	This study	This study
KUZ Z543	Chihaya-akasaka, Osaka, JPN (47)	This study	This study
KUZ Z571	Tsuruga, Fukui, JPN (38)	This study	This study
KUZ Z572	Takashima, Shiga, JPN (39)	This study	This study
KUZ Z573	Takashima, Shiga, JPN (40)	This study	This study
KUZ Z576	Kishiwada, Osaka, JPN (48)	This study	This study
KUZ Z580	Nakanojyo, Gunma, JPN (22)	This study	This study
KUZ Z584	Awajishima, Hyogo, JPN (50)	This study	This study
KUZ Z587	Nantan, Kyoto, JPN (42)	This study	This study
KUZ Z588	Sasayama, Hyogo, JPN (49)	This study	This study
KUZ Z599	Kurobe, Toyama, JPN (27)	This study	This study
KUZ Z601	Toyota, Aichi, JPN (30)	This study	This study
KUZ Z602	Ogaki, Gifu, JPN (35)	This study	This study
KUZ Z607	Izumo, Shimane, JPN (53)	This study	This study
KUZ Z608	Unnan, Shimane, JPN (52)	This study	This study
KUZ Z609	Shobara, Hiroshima, JPN (51)	This study	This study
KUZ Z613	Imabari, Ehime, JPN (54)	This study	This study

Table 2. Continued.

Voucher	Locality (locality no.)	COI	$tRNA^{Cys}$–16S
Orobdella dolichopharynx			
KUZ Z120 (holotype)	Amami-oshima Is., RYK, JPN (71)	AB679680[b]	AB679681[b]
KUZ Z122	Amami-oshima Is., RYK, JPN (69)	AB679682[b]	AB679683[b]
KUZ Z129	Tokunoshima Is., RYK, JPN (73)	This study	This study
KUZ Z567	Kakeromajima Is., RYK, JPN (72)	This study	This study
KUZ Z582	Amami-oshima Is., RYK, JPN (70)	This study	This study
Orobdella esulcata			
KUZ Z29 (holotype)	Kumamoto, Kumamoto, JPN (60)	AB679664[b]	AB679665[b]
KUZ Z170	Ikinoshima Is., Nagasaki, JPN (63)	AB679666[b]	AB679667[b]
KUZ Z556	Hiradojima Is., Nagasaki, JPN (64)	This study	This study
KUZ Z557	Omura, Nagasaki, JPN (62)	This study	This study
KUZ Z590	Futaoijima Is., Yamaguchi, JPN (55)	This study	This study
KUZ Z591	Kitakyushu, Fukuoka, JPN (58)	This study	This study
KUZ Z592	Nakatsu, Oita, JPN (57)	This study	This study
KUZ Z593	Usa, Oita, JPN (56)	This study	This study
KUZ Z598	Ukiha, Fukuoka, JPN (59)	This study	This study
Orobdella ijimai			
KUZ Z110 (topotype)	Nikko, Tochigi, JPN (15)	AB679672[b]	AB679673[b]
KUZ Z188	Saku, Nagano, JPN (24)	AB679674[b]	AB679675[b]
KUZ Z549	Nakanojyo, Gunma, JPN (23)	This study	This study
KUZ Z585	Daigo, Ibaraki, JPN (13)	This study	This study
KUZ Z604	Kasama, Ibaraki, JPN (14)	This study	This study
[Orobdella kawakatsuorum			
KUZ Z148	Rishirito Is., Hokkaido, JPN (8)	AB679692[b]	AB679693[b]
KUZ Z150	Kamishihoro, Hokkaido, JPN (9)	AB679694[b]	AB679695[b]
KUZ Z152	Shari, Hokkaido, JPN (1)	AB679696[b]	AB679697[b]
KUZ Z153	Ashoro, Hokkaido, JPN (2)	AB679698[b]	AB679699[b]
KUZ Z154	Kamikawa, Hokkaido, JPN (4)	AB679700[b]	AB679701[b]
KUZ Z159	Kyowa, Hokkaido, JPN (12)	AB679702[b]	AB679703[b]
KUZ Z167 (topotype)	Sapporo, Hokkaido, JPN (11)	This study	This study

Table 2. Continued.

Voucher	Locality (locality no.)	COI	$tRNA^{Cys}$–16S
Orobdella ketagalan			
KUZ Z208 (holotype)	New Taipei City, TWN (82)	AB704787[c]	This study
Orobdella koikei			
KUZ Z145	Hiratori, Hokkaido, JPN (7)	AB679684[b]	AB679685[b]
KUZ Z146	Hidaka, Hokkaido, JPN (6)	AB679686[b]	AB679687[b]
KUZ Z151	Shari, Hokkaido, JPN (1)	This study	This study
KUZ Z156 (holotype)	Kamikawa, Hokkaido, JPN (4)	AB679688[b]	AB679689[b]
KUZ Z158	Mashike, Hokkaido, JPN (10)	AB679690[b]	AB679691[b]
Orobdella mononoke			
KUZ Z223	Yakushima Is., RYK, JPN (68)	AB698864[a]	AB698865[a]
KUZ Z224 (holotype)	Yakushima Is., RYK, JPN (68)	AB698866[a]	AB698867[a]
Orobdella octonaria			
KUZ Z177	Akiruno, Tokyo, JPN (17)	AB679706[b]	AB679707[b]
KUZ Z181 (topotype)	Hakone, Kanagawa, JPN (20)	AB679708[b]	AB679709[b]
KUZ Z226	Taiki, Mie, JPN (45)	This study	This study
KUZ Z535	Oume, Tokyo, JPN (16)	This study	This study
KUZ Z537	Nishihara, Yamanashi, JPN (18)	This study	This study
KUZ Z547	Ono, Yamanashi, JPN (19)	This study	This study
KUZ Z551	Nakatsugawa, Gifu, JPN (28)	This study	This study
KUZ Z552	Shizuoka, Shizuoka, JPN (25)	This study	This study
KUZ Z554	Shinshiro, Aichi, JPN (29)	This study	This study
KUZ Z579	Chihaya-askasaka, Osaka, JPN (47)	This study	This study
KUZ Z586	Izu, Shizuoka, JPN (21)	This study	This study
Orobdella shimadae			
KUZ Z128 (holotype)	Okinawajima Is., RYK, JPN (74)	AB679676[b]	AB679677[b]
KUZ Z131	Okinawajima Is., RYK, JPN (76)	This study	This study
KUZ Z138	Okinawajima Is., RYK, JPN (78)	AB679678[b]	AB679679[b]
KUZ Z558	Okinawajima Is., RYK, JPN (75)	This study	This study
KUZ Z560	Okinawajima Is., RYK, JPN (77)	This study	This study
KUZ Z562	Okinawajima Is., RYK, JPN (79)	This study	This study

Table 2. Continued.

Voucher	Locality (locality no.)	COI	$tRNA^{Cys}$–16S
Orobdella shimadae (continued)			
KUZ Z563	Okinawajima Is., RYK, JPN (80)	This study	This study
KUZ Z566	Okinawajima Is., RYK, JPN (81)	This study	This study
Orobdella tsushimensis			
KUZ Z133	Tsushima Is., Nagasaki, JPN (65)	AB679660[b]	AB679661[b]
KUZ Z134 (holotype)	Tsushima Is., Nagasaki, JPN (66)	AB679662[b]	AB679663[b]
KUZ Z215	Gageodo Is., Jeollanam-do, KOR (67)	AB693165[d]	AB693166[d]
Outgroup			
Erpobdella japonica (Erpobdellidae)			
KUZ Z178	Saku, Nagano, JPN	AB679654[b]	AB679655[b]
Gastrostomobdella monticola (Gastrostomobdellidae)			
UNIMAS/A3/BH01/10	Kuching, Borneo, MYS	AB679656[b]	AB679657[b]
Mimobdella japonica (Salifidae)			
KUZ Z179	Amami-oshima Is., RYK, JPN	AB679658[b]	AB679659[b]

Acronym: UNIMAS, The Universiti Malaysia Sarawak. Abbreviations: KOR, Korea; JPN, Japan; RYK, Ryukyu Islands; MYS, Malaysia; TWN, Taiwan. Sources: [a]Nakano (2012a), [b]Nakano (2012c), [c]Nakano & Lai (2012), [d]Nakano & Seo (2012).

Table 3. Comparison of morphological characters among six quadrannulate *Orobdella* species.

Character	*O. whitmani*	*O. esulcata*	*O. kawakatsuorum*	*O. ketagalan*	*O. koikei*	*O. tsushimensis*
Somite IV	uniannulate	uniannulate	biannulate	uniannulate	uniannulate	uniannulate
Somite XXVI	quadrannulate	triannulate	triannulate	triannulate	dorsally triannulate, ventrally biannulate	triannulate
Number of annuli between gonopores	1/2 + 4 + 1/2	1/2 + 4 + 1/2	6	1/2 + 4 + 1/2	1/2 + 4 + 1/2	1/2 + 5
Gastroporal duct	bulbous	tubular, but slightly bulbous at junction with gastropore	simple tubular	simple tubular	tubular, but bulbous at junction with crop	bulbous
Epididymides	middle of XV to posterior of XVIII, occupying 6–11 annuli	middle of XV to middle of XX, occupying 10–15 annuli	middle of XVI to posterior of XVII, occupying 1–2 annuli	absent	anterior of XVI to anterior of XIX, occupying 9–12 annuli	posterior of XVI to posterior of XIX, occupying 8–10 annuli
Paired sperm duct bulbs	absent	absent	absent	in XV, occupying 1–2 annuli	absent	absent
Ejaculatory ducts anterior to ovisacs	coiled	loosely coiled	nearly straight	loosely coiled	nearly straight	straight
Atrial cornua	muscular, ovate	muscular, ovate	undeveloped, conical	undeveloped, conical	muscular, ovate	muscular, ovate

Table 4. Comparison of morphological characters among four sexannulate *Orobdella* species.

Character	*O. dolichopharynx*	*O. ijimai*	*O. mononoke*	*O. shimadae*
Somite III	uniannulate	biannulate	uniannulate	uniannulate
Somite IV	biannulate	biannulate	uniannulate	biannulate
Somite VI	triannulate	triannulate	dorsally quadrannulate, ventrally triannulate	triannulate
Somite VII	quadrannulate	quadrannulate	quadrannulate	triannulate
Somite VIII	quinquannulate	sexannulate	sexannulate	quinquannulate
Somite XXVI	sexannulate	quadrannulate	quinquannulate	quinquannulate
Number of annuli between gonopores	8	1/2 + 7 + 1/2	8 + 1/2	9
Pharynx	reaching to XVI	reaching to XIV	reaching to XIV	reaching to XVI
Gastroporal duct	rudimentary tubular, reaching to XVI	bulbous	tubular, but slightly bulbous at junction with crop	rudimentary tubular, reaching to XV
Epididymides	absent	posterior of XVI to posterior of XIX, occupying 11–17 annuli	middle of XV to posterior of XIX, occupying 20–22 annuli	absent
Ejaculatory ducts anterior to ovisacs	coiled	nearly straight	loosely coiled	coiled
Pre-atrial loop	extending to middle of XI	absent	absent	extending to middle of XI
Atrial cornua	absent	muscular, ovate	muscular, ovate	absent

Figures

Figure legends

Fig. 1. Map showing collection localities for *Orobdella* species. For the names of these localities, see Table 2 and Appendix.

Fig. 2. The ML tree modified from figure 1 in Nakano *et al*. (2012). Asterisks indicate nodes with ML bootstrap values higher than 70% and Bayesian posterior probabilities higher than 95%.

Fig. 3. Schematic drawings of the gastroporal duct and genital organs of *Orobdella* and *Gastrostomobdella*, modified from figure 2 in Nakano *et al*. (2012). A, *Orobdella whitmani*; B, *Gastrostomobdella monticola*. Abbreviations: cp, crop; gd, gastroporal duct; gp, gastropore; nv, nerve cord; o, ovisac; ph, pharynx; sd, sperm duct.

Fig. 4. *Orobdella whitmani* Oka, 1895 from the type locality, Mt. Kinkazan, Gifu, Gifu, Japan, KUZ Z45. A, dorsal view; B, ventral view.

Fig. 5. *Orobdella whitmani* Oka, 1895, *O*. *dolichopharynx* Nakano, 2011, and *O*. *esulcata* Naknao, 2010, photographs of live animals, dorsal views. A, *O*. *whitmani* from Kurobe, Toyama, Japan, KUZ Z599; B, *ditto*, from Shobara, Hiroshima, Japan, KUZ Z609; C, *O*. *dolichopharynx* from Amami-oshima, Ryukyu Islands, Japan, KUZ Z567; D, *O*. *esulcata*, from Ikinoshima, Nagasaki, Japan, KUZ Z176; E, *ditto*, from Usa, Oita, Japan, KUZ Z593; F, *ditto*, from Ukiha, Fukuoka, Japan, KUZ Z598.

Fig. 6. *Orobdella whitmani* Oka, 1895, from the type locality, Mt. Kinkazan, Gifu, Gifu, Japan, KUZ Z45, modified from figure 3 in Nakano (2010). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; an, anus; at, atrium; cod, common

oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 7. *Orobdella dolichopharynx* Nakano, 2011, holotype, KUZ Z120, modified from figure 5 in Nakano (2011a). A, dorsal view; B, ventral view.

Fig. 8. *Orobdella dolichopharynx* Nakano, 2011, holotype, KUZ Z120, modified from figure 5 in Nakano (2011a). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium showing position of ganglion XI; J, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: af, annular furrow; an, anus; at, atrium; cl, clitellum; cod, common oviduct; cp, crop; fp, female gonopore; gd, gastroporal duct; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; sd, sperm duct; t, testisac.

Fig. 9. *Orobdella esulcata* Nakano, 2010, from Ukiha, Fukuoka, Japan, KUZ Z598. A, dorsal view; B, ventral view.

Fig. 10. *Orobdella esulcata* Nakano, 2010, holotype, KUZ Z29, modified from figure 6 in Nakano (2010). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 11. *Orobdella ijimai* Oka, 1895, from the type locality, Nikko, Tochigi, Japan, KUZ Z108, modified from figure 2 in Nakano (2011a). A, dorsal view; B, ventral view.

Fig. 12. *Orobdella ijimai* Oka, 1895, *O. kawakatsuorum* Richardson, 1975, *O. koikei* Nakano, 2012, and *O. mononoke* Naknao, 2012, photographs of live animals, dorsal views. A, *O. ijimai* from Daigo, Ibaraki, Japan, KUZ Z585; B, *O. kawakatsuorum* from near the type locality, Sapporo, Hokkaido, Japan, KUZ Z167; C, *O. koikei*, holotype, KUZ Z156; D, *O. mononoke*, holotype, KUZ Z224.

Fig. 13. *Orobdella ijimai* Oka, 1895, from the type locality, Nikko, Tochigi, Japan, KUZ Z108, modified from figure 3 in Nakano (2011a). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 14. *Orobdella kawakatsuorum* Richardson, 1975, from near the type locality, Sapporo, Hokkaido, Japan, KUZ Z167, modified from figure 7 in Nakano (2012c). A, dorsal view; B, ventral view.

Fig. 15. *Orobdella kawakatsuorum* Richardson, 1975, from near the type locality, Sapporo, Hokkaido, Japan, KUZ Z167, A–G modified from figure 10, and H–L from figure 11 in Nakano (2012c). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male

atrium; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: af, annular furrow; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 16. *Orobdella ketagalan* Nakano & Lai, 2012, holotype, KUZ Z208, modified from figure 2 in Nakano & Lai (2012). A, dorsal view; B, ventral view.

Fig. 17. *Orobdella ketagalan* Nakano & Lai, 2012, holotype, KUZ Z208, A–G modified from figure 3, and H–M from figure 4 in Nakano & Lai (2012). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, lateral view of bulb of right sperm duct; J, dorsal view of male atrium showing position of ganglion XI; K, lateral view of male atrium; L, ventral view of male atrium; M, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: an, anus; at, atrium; cl, clitellum; cod, common oviduct; cp, crop; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; sd, sperm duct; sdb, sperm duct bulb; t, testisac.

Fig. 18. *Orobdella koikei* Nakano, 2012, holotype, KUZ Z156, modified from figure 2 in Nakano (2012c). A, dorsal view; B, ventral view.

Fig. 19. *Orobdella koikei* Nakano, 2012, holotype, KUZ Z156, A–G modified from figure 3, and H–L from figure 4 in Nakano (2012c). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium; J, lateral view of male atrium; K, ventral view of male

atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; af, annular furrow; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 20. *Orobdella mononoke* Nakano, 2012, holotype, KUZ Z224, modified from figure 3 in Nakano (2012a). A, dorsal view; B, ventral view.

Fig. 21. *Orobdella mononoke* Nakano, 2012, holotype, KUZ Z224, A–G modified from figure 4, and H–L from figure 5 in Nakano (2012a). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium showing position of ganglion XI; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 22. *Orobdella octonaria* Oka, 1895, from the type locality, KUZ Z181, modified from figure 5 in Nakano (2012b). A, dorsal view; B, ventral view.

Fig. 23. *Orobdella octonaria* Oka, 1895, *O. shimadae* Nakano, 2011, and *O. tsushimensis* Naknao, 2011, photographs of live animals, dorsal views. A, *O. octonaria*, from the type locality, Mt. Yusakayama, Hakone, Kanagawa, Japan, KUZ Z181; B, *ditto*, from Shinshiro, Aichi, Japan, KUZ Z554; C, *ditto*, from Chihaya-akasaka, Osaka, Japan, KUZ Z579; D, *ditto*, from Izu, Shizuoka, Japan, KUZ Z586; E, *O. shimadae* from Okinawajima Island, Ryukyu Islands, Japan, KUZ Z558; F, *O. tsushimensis*, from Tsushima Island, Nagasaki, Japan, KUZ Z173.

Fig. 24. *Orobdella octonaria* Oka, 1895. A–E, from the type locality, Mt. Yusakayama, Hakone, Japan, KUZ Z181; F–L, lectotype, NSMT-An 415. A–E modified from figure 7, F, G from figure 3, and H–L from figure 4 in Nakano (2012b). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium showing position of ganglion XI; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx.

Fig. 25. *Orobdella shimadae* Nakano, 2011 holotype, KUZ Z128, modified from figure 8 in Nakano (2011a). A, dorsal view; B, ventral view.

Fig. 26. *Orobdella shimadae* Nakano, 2011, holotype, KUZ Z128, modified from figure 8 in Nakano (2011a). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium showing position of ganglion XI; J, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: af, annular furrow; an, anus; at, atrium; cl, clitellum; cod, common oviduct; cp, crop; fp, female gonopore; gd, gastroporal duct; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; sd, sperm duct.

Fig. 27. *Orobdella tsushimensis* Nakano, 2011, holotype, KUZ Z134, modified from figure 2 in Nakano (2011b). A, dorsal view; B, ventral view.

Fig. 28. *Orobdella tsushimensis* Nakano, 2011, holotype, KUZ Z134, A–G modified from figure 3, and H–L from figure 4 in Nakano (2011b). A, dorsal view of somites

I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXV–XXVII and caudal sucker; D, ventral view of somites XXV–XXVII and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastroporal duct; G, ventral view of gastropore and female gonopore; H, dorsal view of reproductive system and ventral nervous system; I, dorsal view of male atrium; J, lateral view of male atrium; K, ventral view of male atrium; L, dorsal view of female reproductive system showing position of ganglion XIII. Abbreviations: ac, atrial cornu; af, annular furrow; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testisac.

Fig. 29. The ML tree of 2008 bp of COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$, and 16S rRNA. Species names in red indicate quadrannulate; green, sexannulate; and blue, octannulate. The numbers associated with the nodes represent the bootstrap value (BS)/and Bayesian posterior probabilities (BPPs). BSs higher than 70% and/or BPPs higher than 95% are indicated. For the locality number, see Figure 1 and Table 2.

Fig. 30. The distribution range of each species of *Orobdella*. Species names in red indicate quadrannulate; green, sexannulate; and blue, octannulate. For the clade name, see Figure 29.

Fig. 1.


Hokkaido
Honshu
Shikoku
Kyushu
Tsushima Is.
Gageodo Is.
Yakushima Is.
Amami-oshima Is.
Tokunoshima Is.
Okinawajima Is.
Taiwan

Fig. 2.

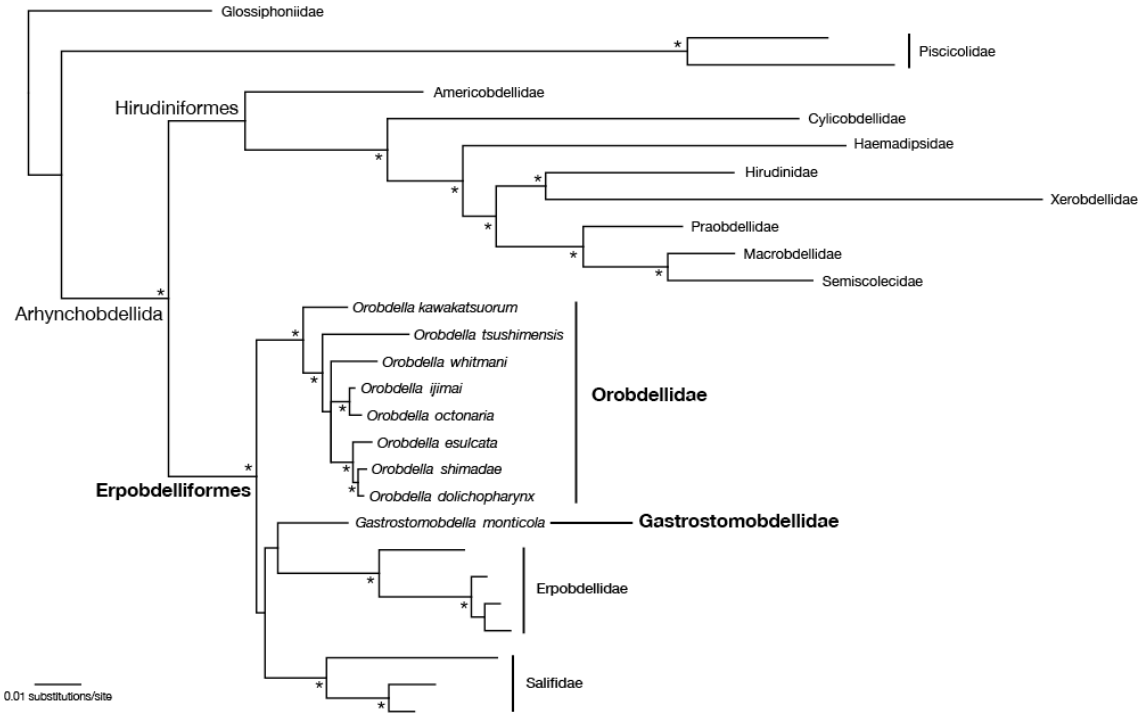

Glossiphoniidae
Piscicolidae
Americobdellidae
Hirudiniformes
Cylicobdellidae
Haemadipsidae
Hirudinidae
Xerobdellidae
Praobdellidae
Macrobdellidae
Semiscolecidae
Arhynchobdellida
Orobdella kawakatsuorum
Orobdella tsushimensis
Orobdella whitmani
Orobdella ijimai
Orobdella octonaria
Orobdella esulcata
Orobdella shimadae
Orobdella dolichopharynx
Orobdellidae
Erpobdelliformes
Gastrostomobdella monticola
Gastrostomobdellidae
Erpobdellidae
Salifidae
0.01 substitutions/site

Fig. 3.

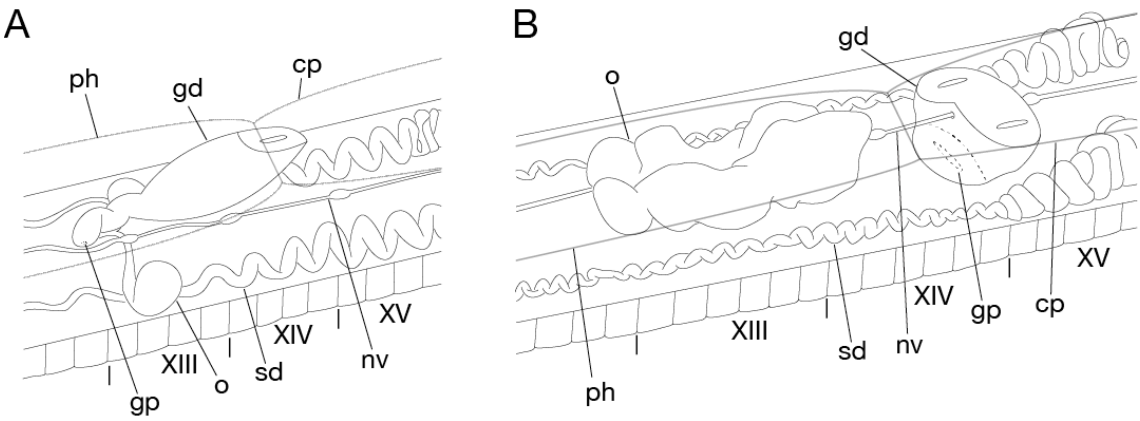
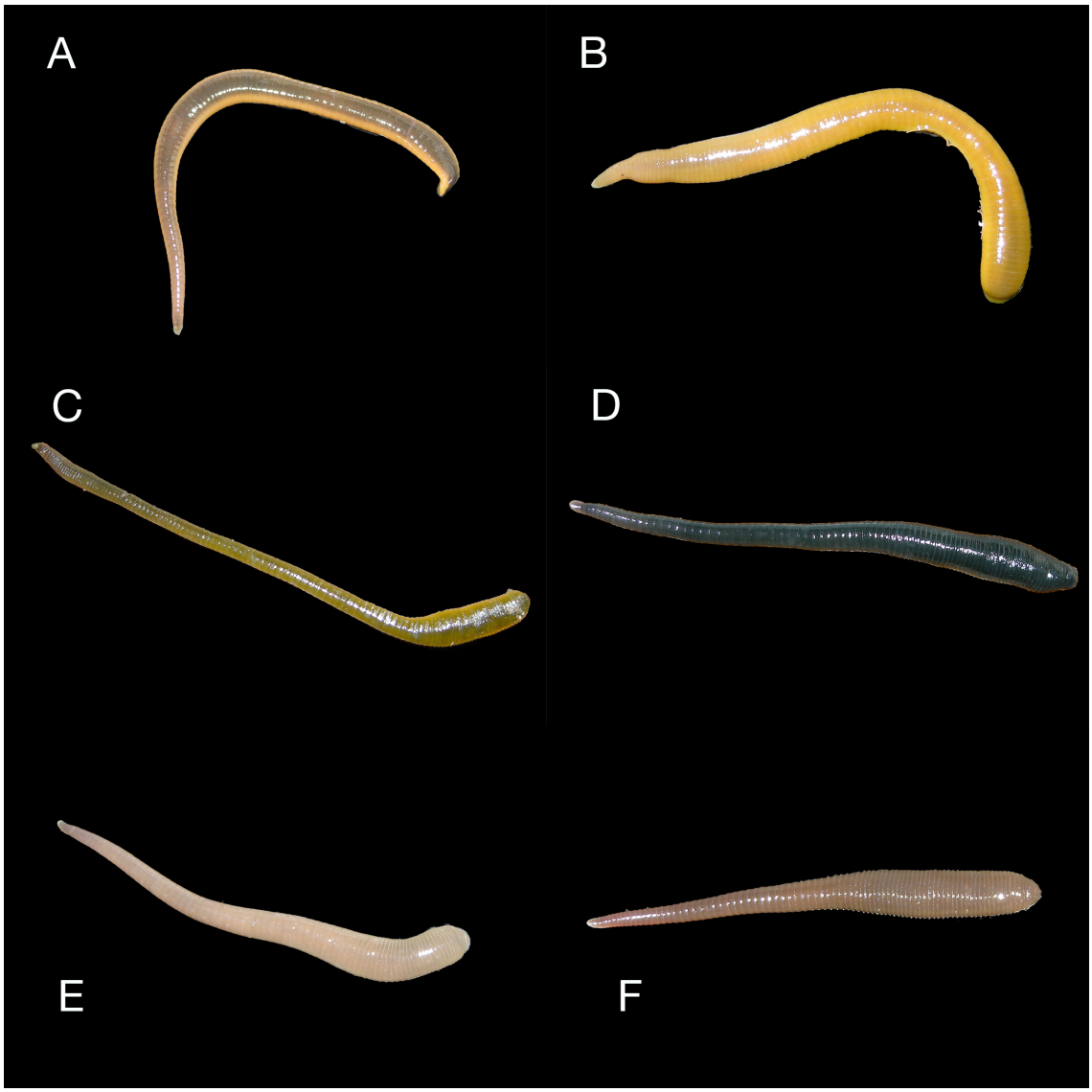


Fig. 4.

Fig. 5.



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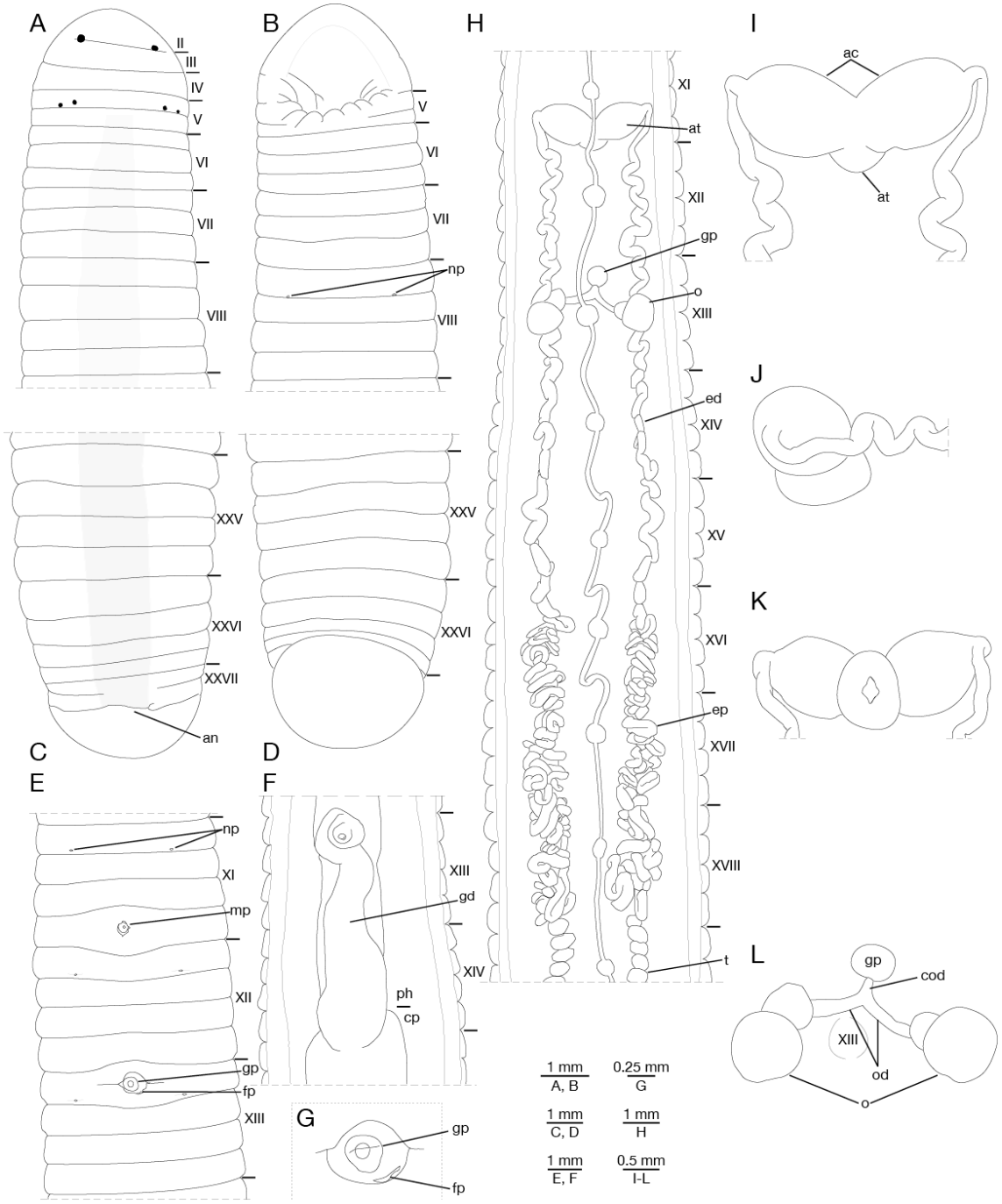
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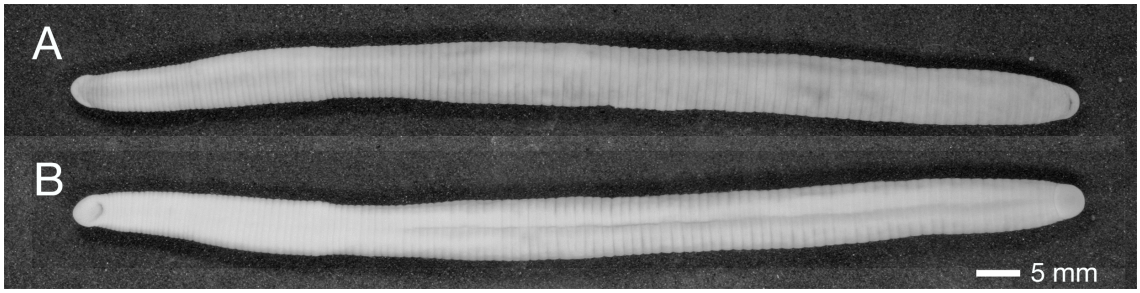
Fig. 7.



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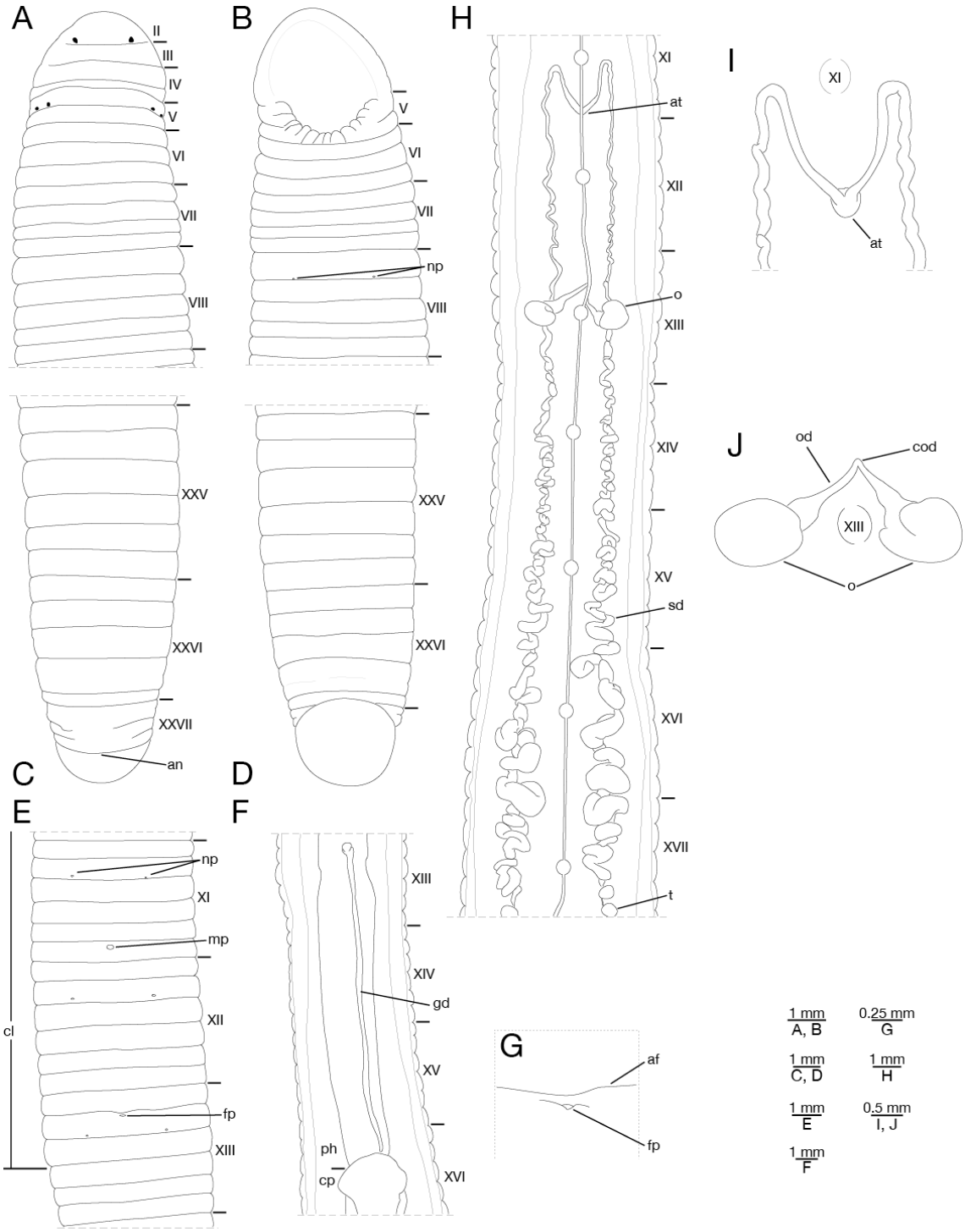
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Fig. 9.

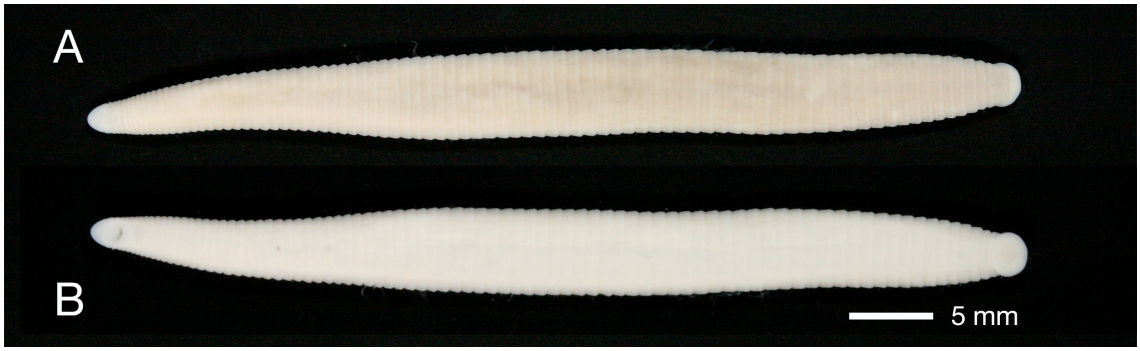

Fig. 10.

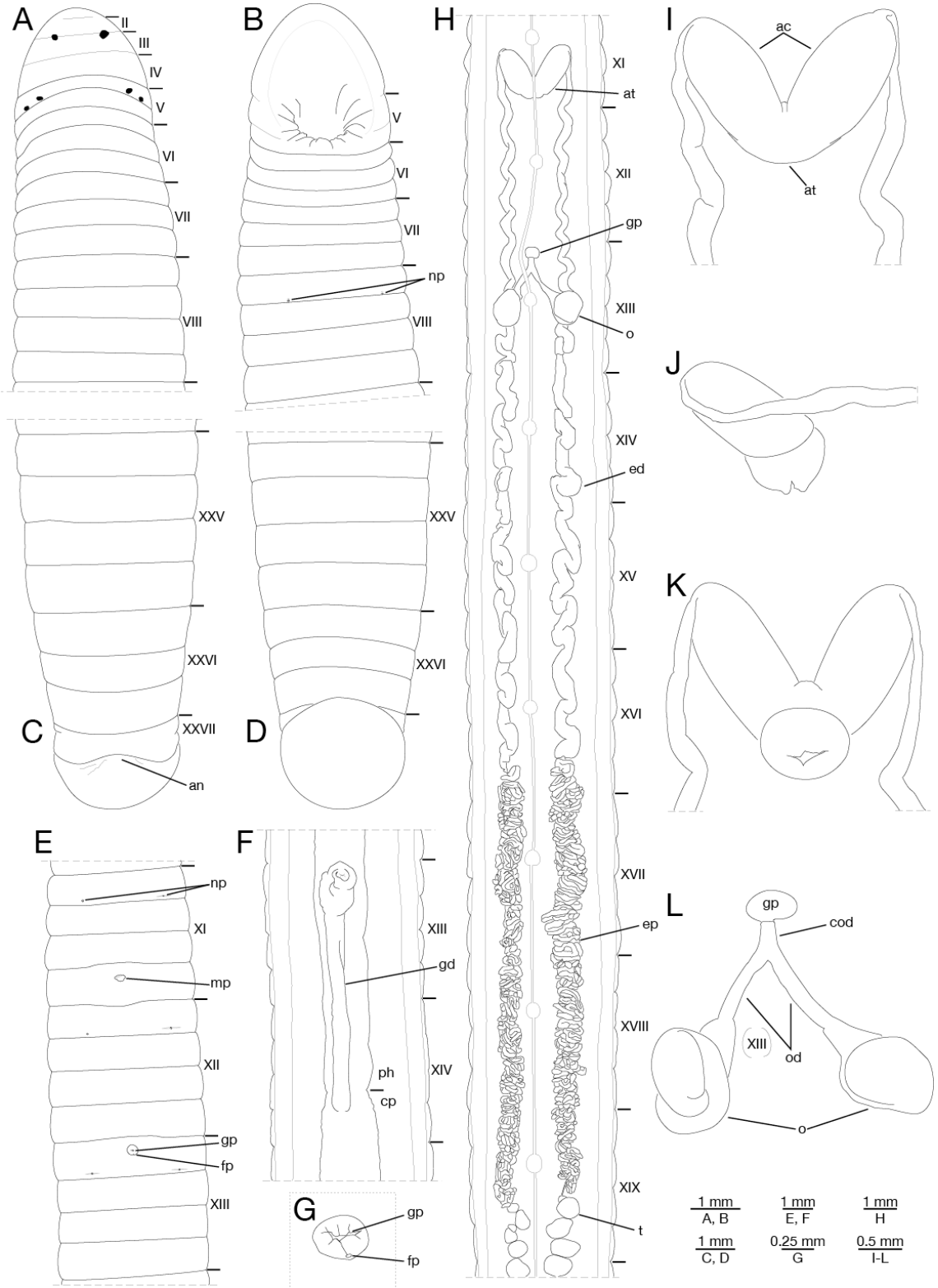
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Fig. 11.

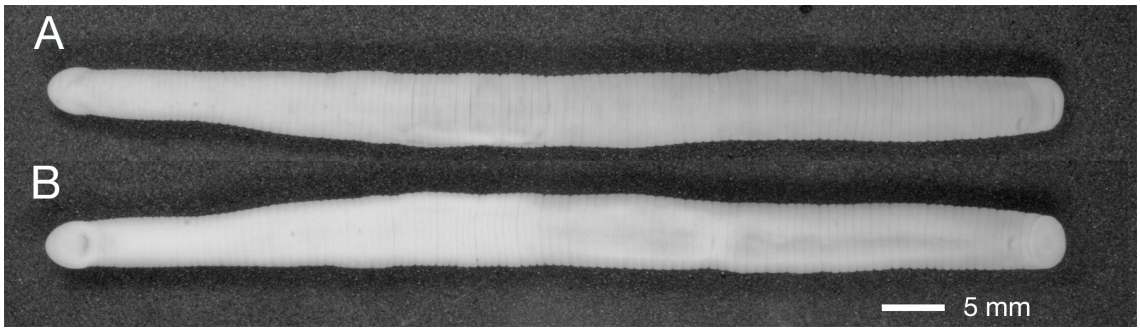

Fig. 12.

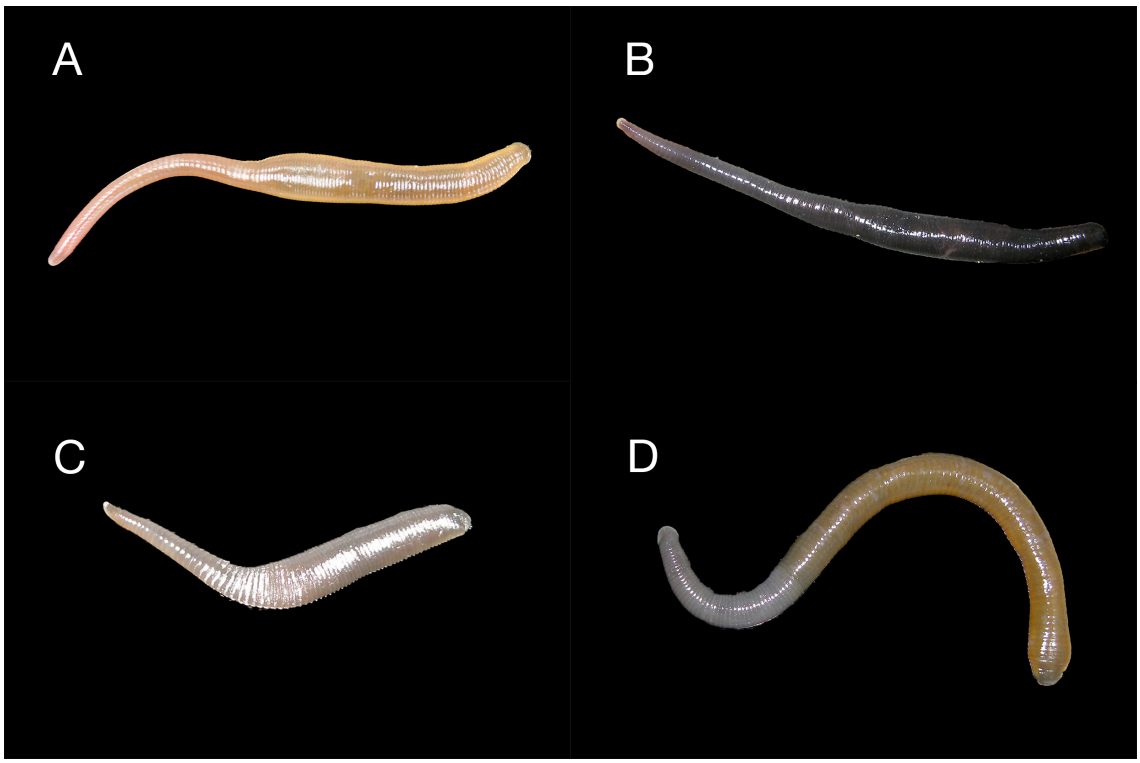

Fig. 13.

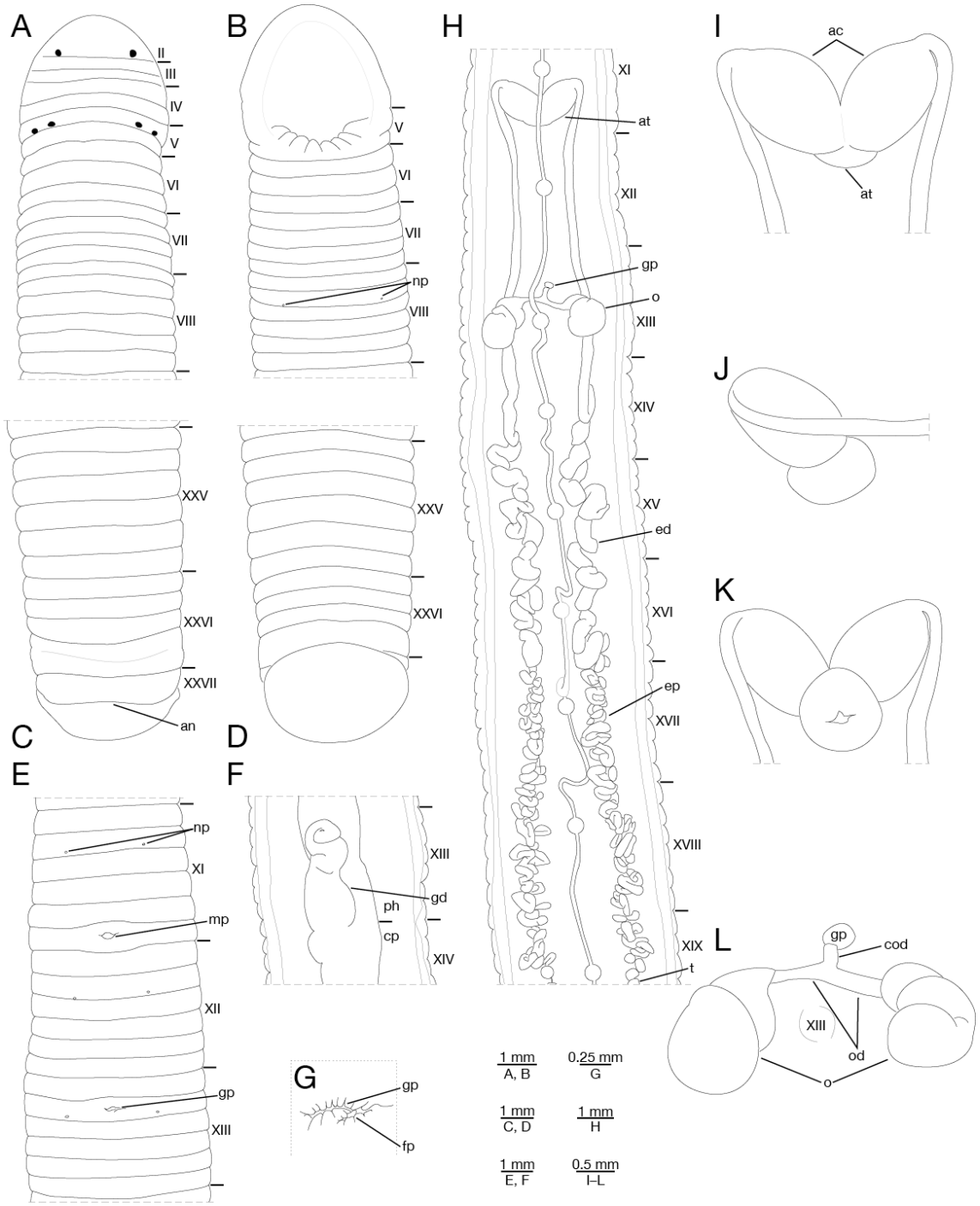
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Fig. 14.

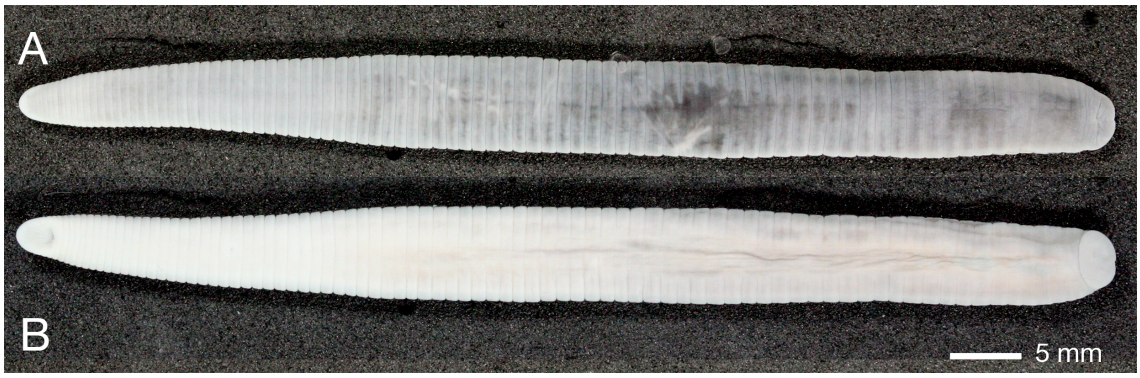
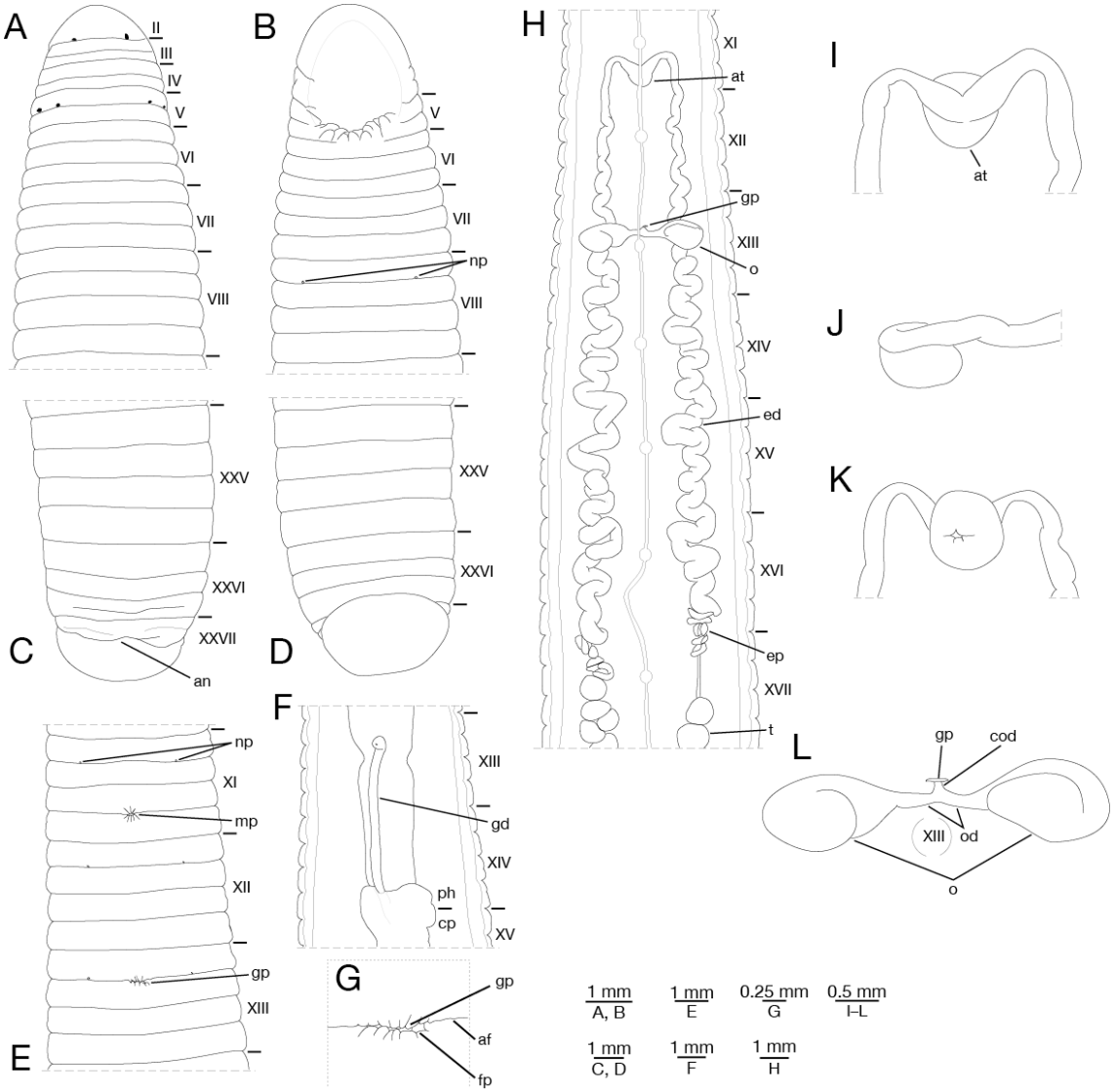


Fig. 15.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K
L
gp
cod
XIII
od
o
1 mm
A, B
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E
0.25 mm
G
0.5 mm
I–L
1 mm
C, D
1 mm
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1 mm
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Fig. 16.

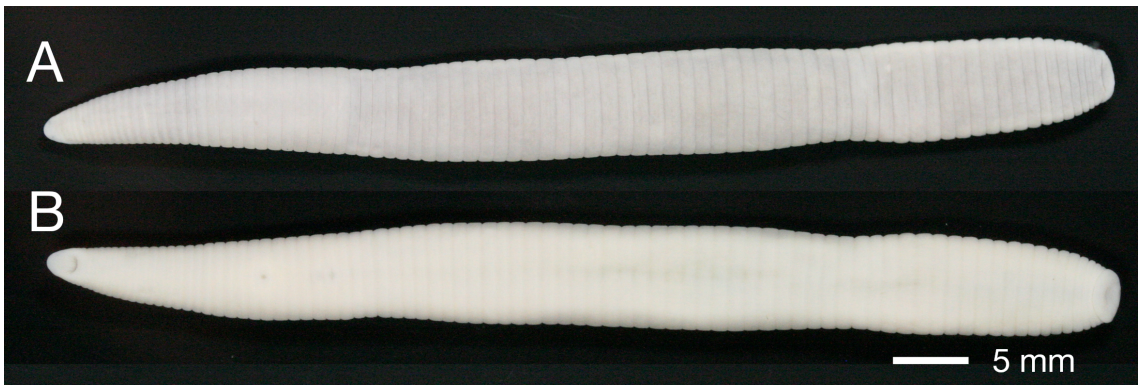
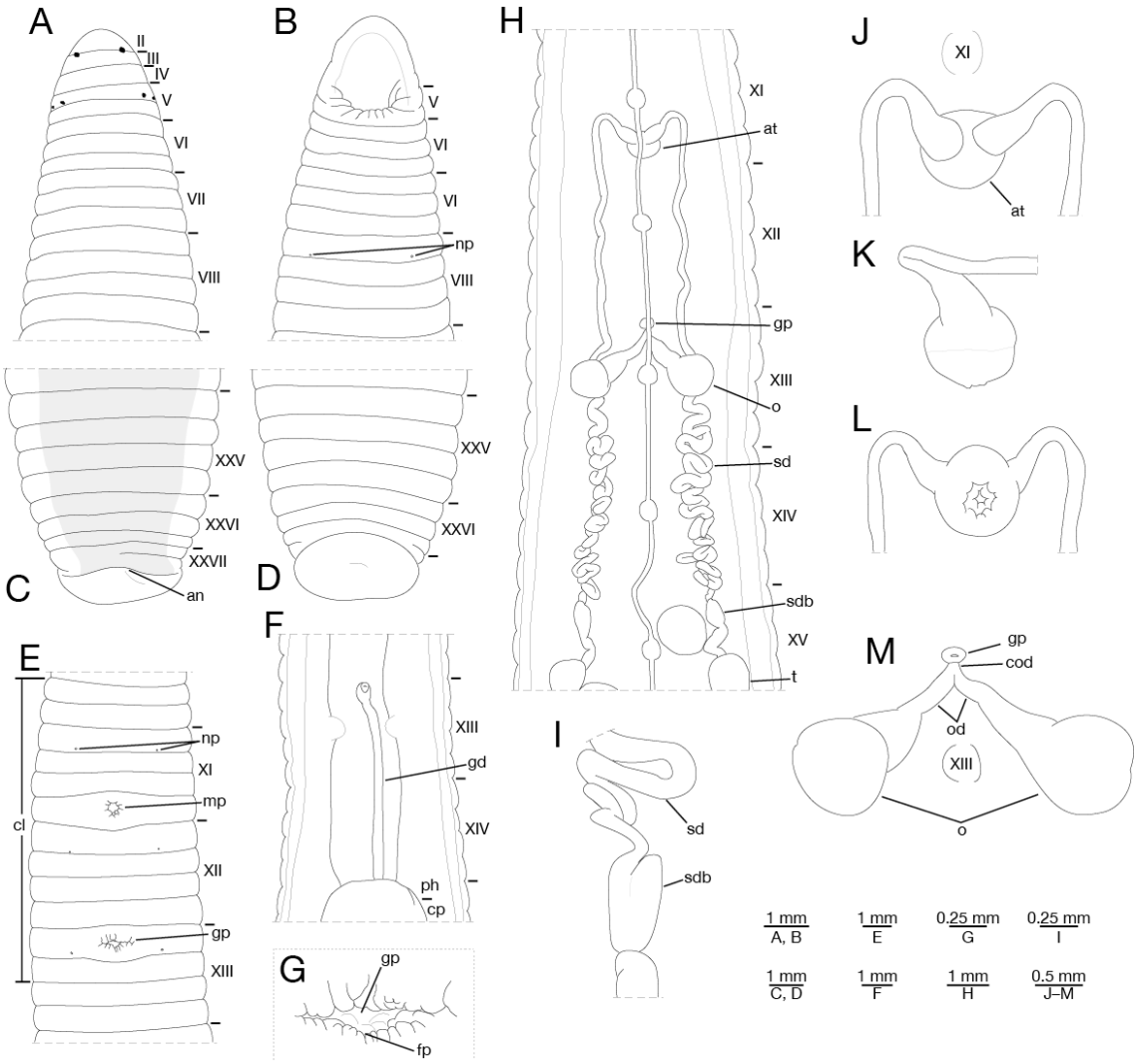


Fig. 17.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XV
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XI
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1 mm
A, B
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1 mm
C, D
1 mm
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0.5 mm
J–M

Fig. 18.

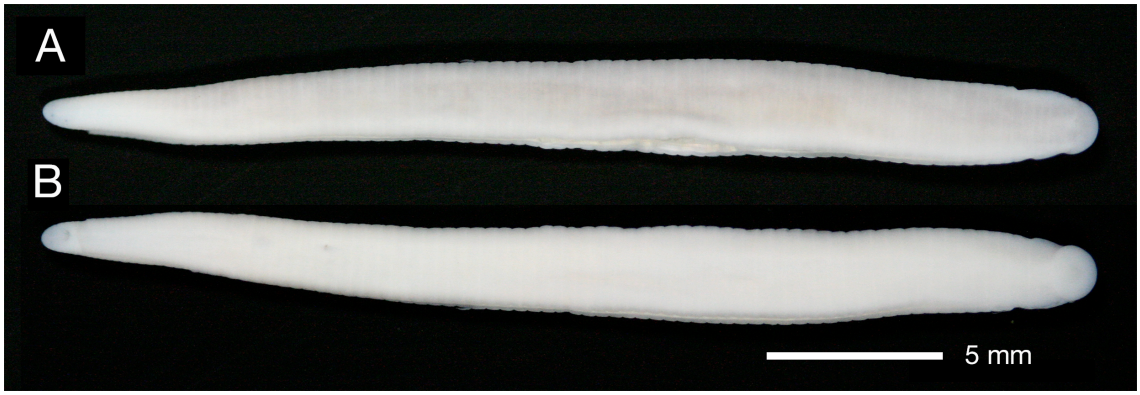
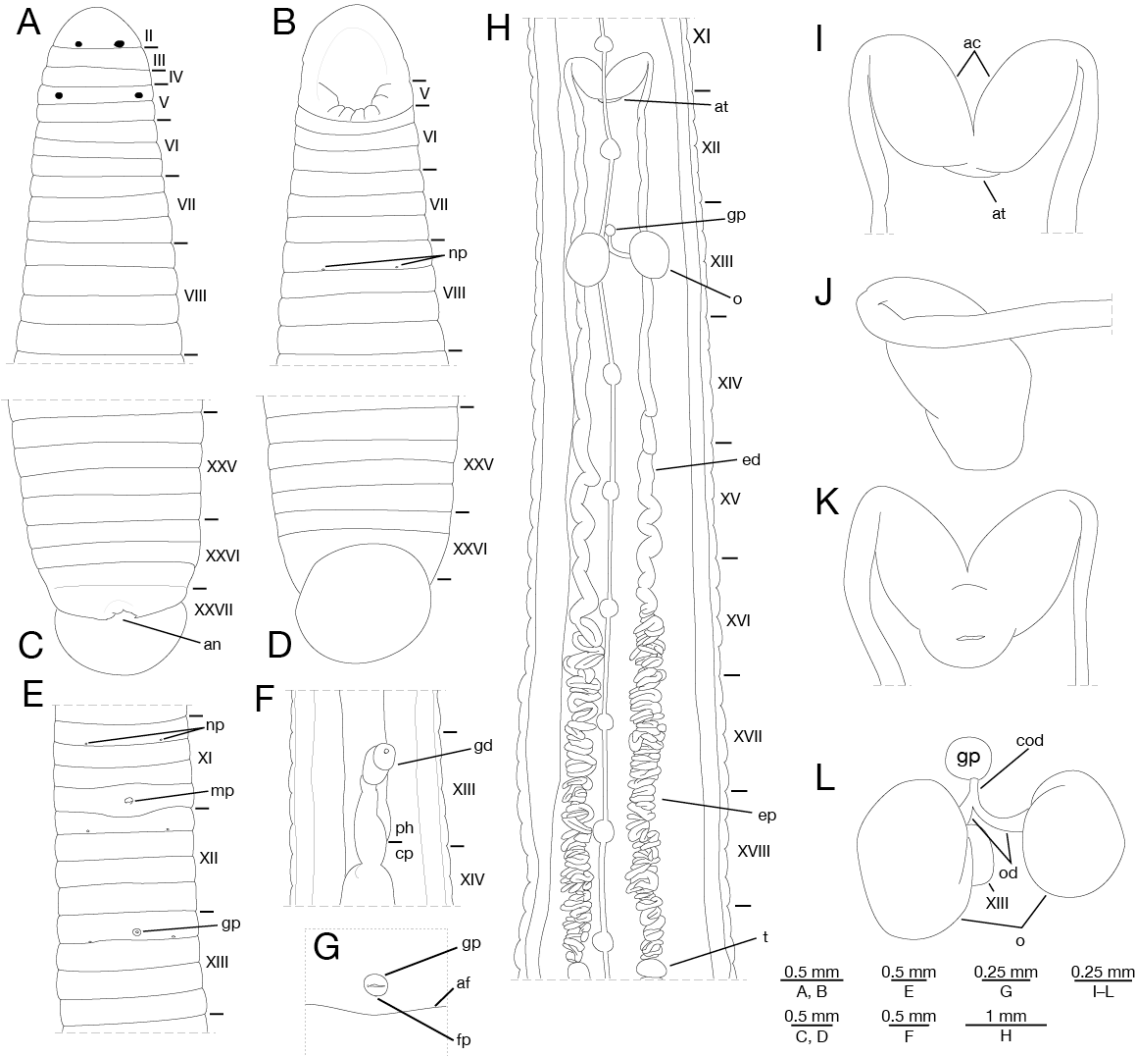


Fig. 19.


A
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cod
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XIII
o
0.5 mm
A, B
0.5 mm
E
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G
0.25 mm
I–L
0.5 mm
C, D
0.5 mm
F
1 mm
H

Fig. 20.

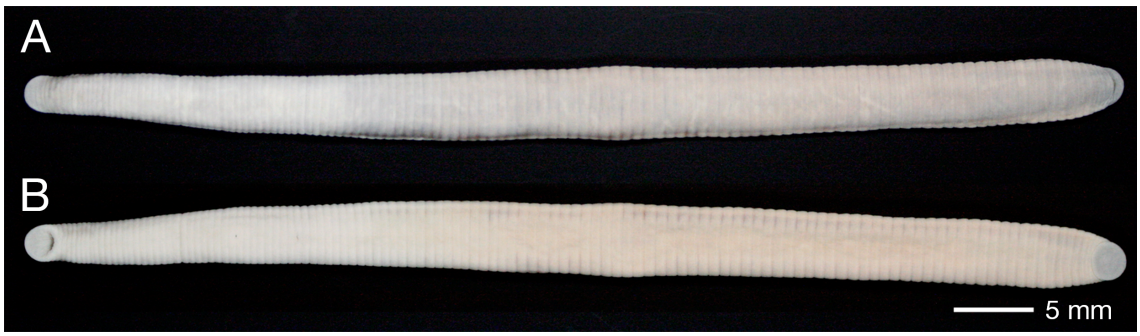
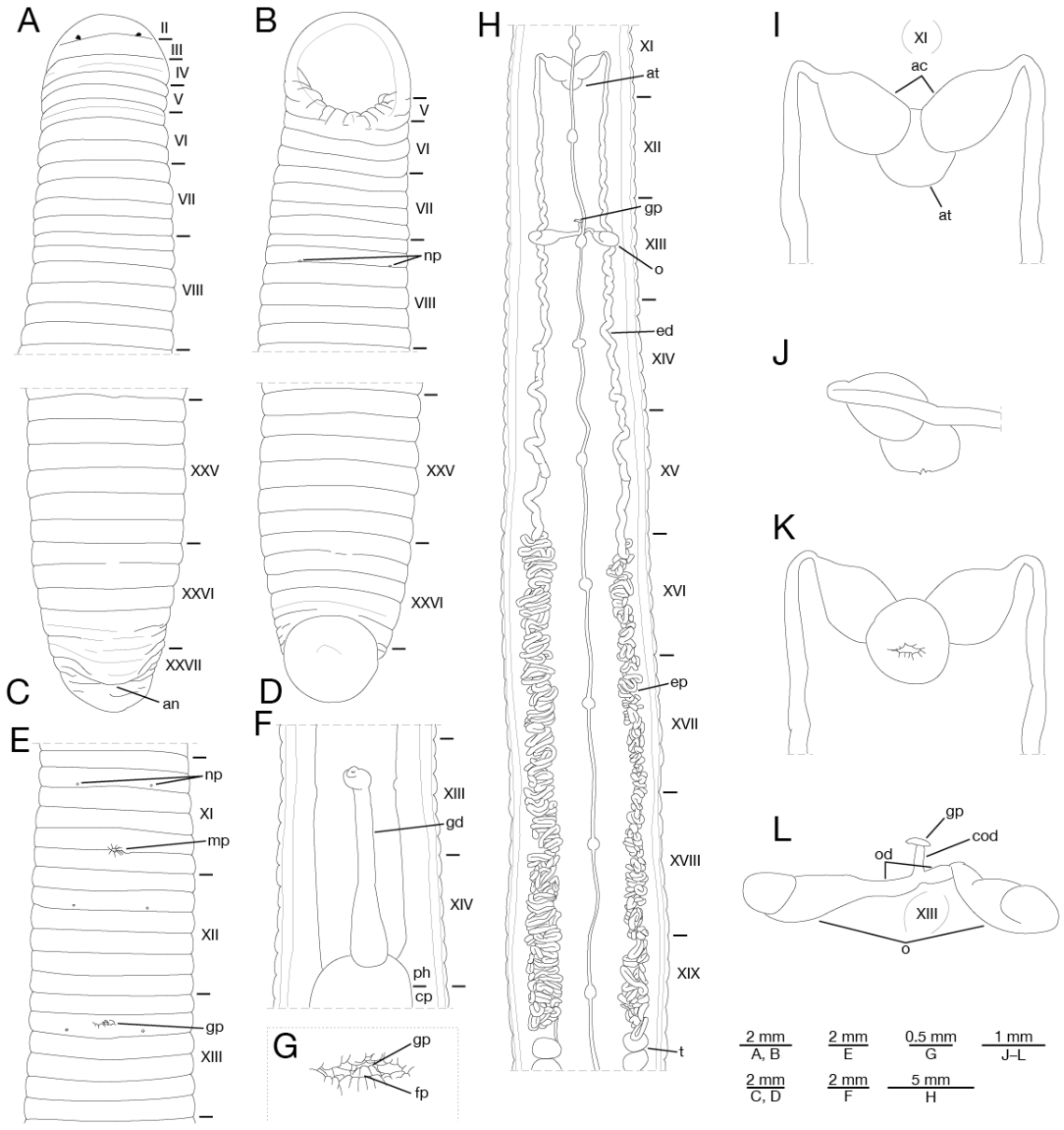


Fig. 21.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cod
od
XIII
o
2 mm
A, B
2 mm
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0.5 mm
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1 mm
J–L
2 mm
C, D
2 mm
F
5 mm
H

Fig. 22.

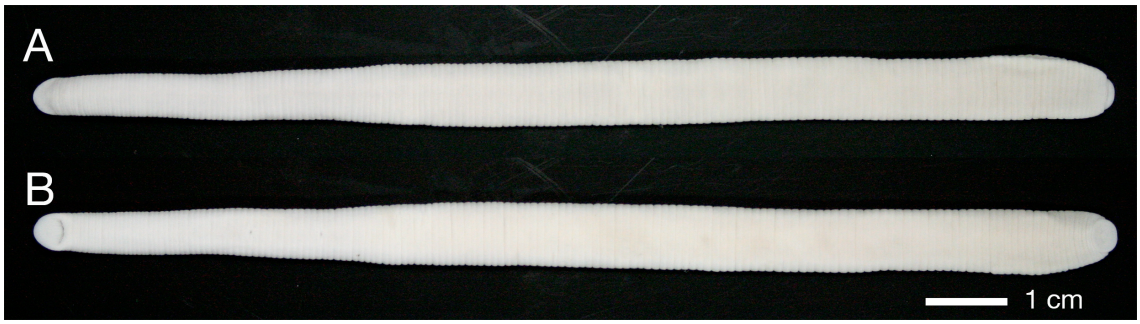

Fig. 23.

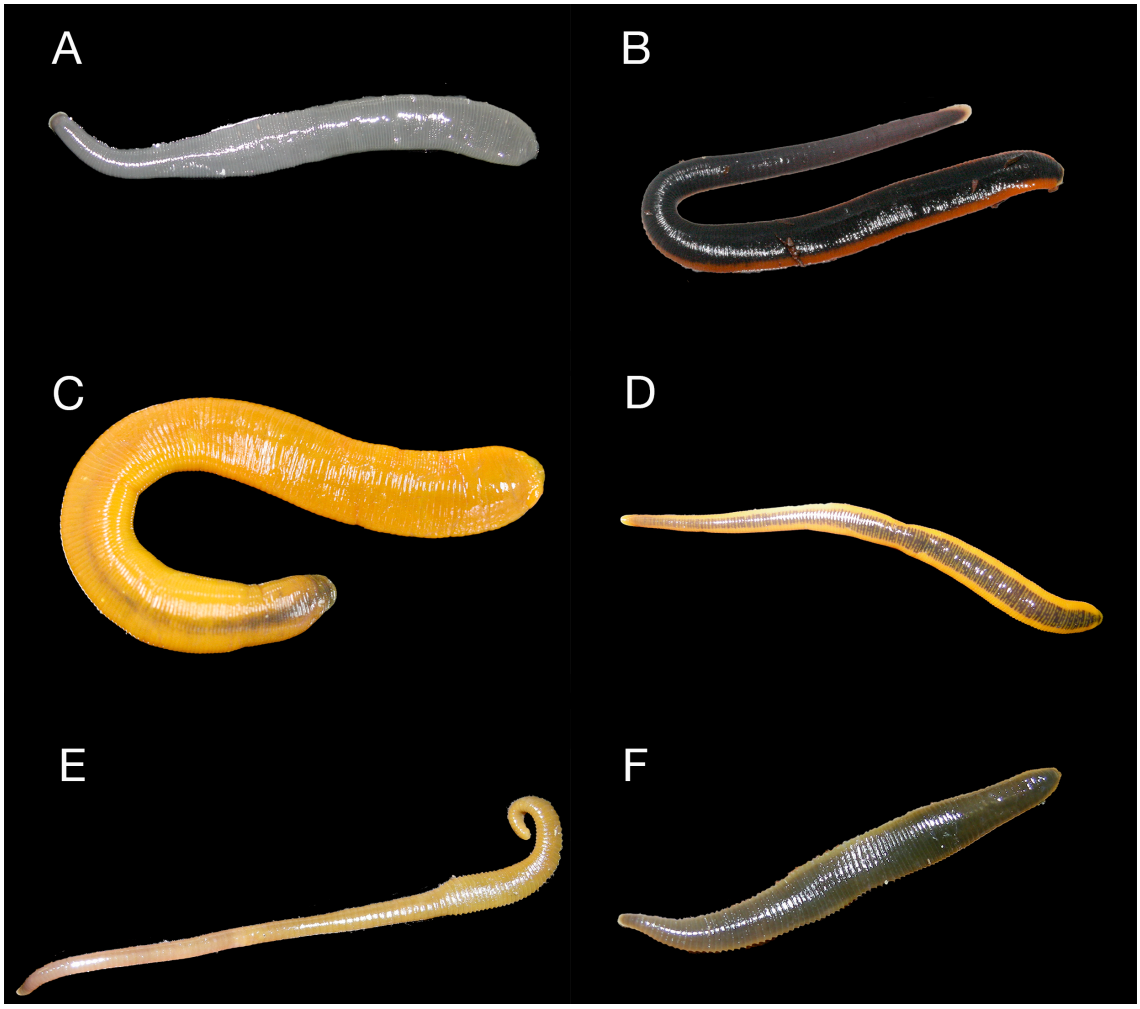

Fig. 24.

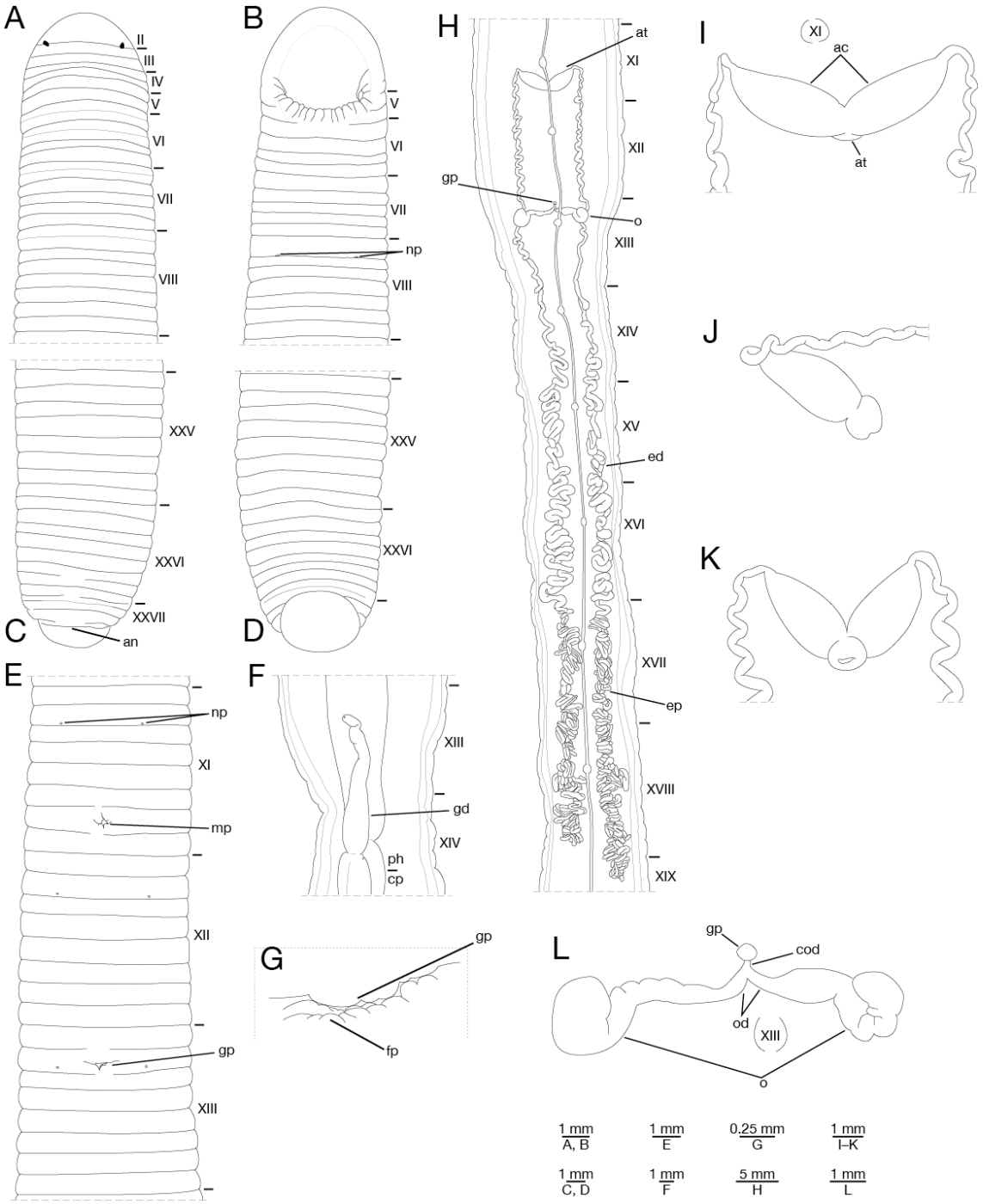
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Fig. 25.

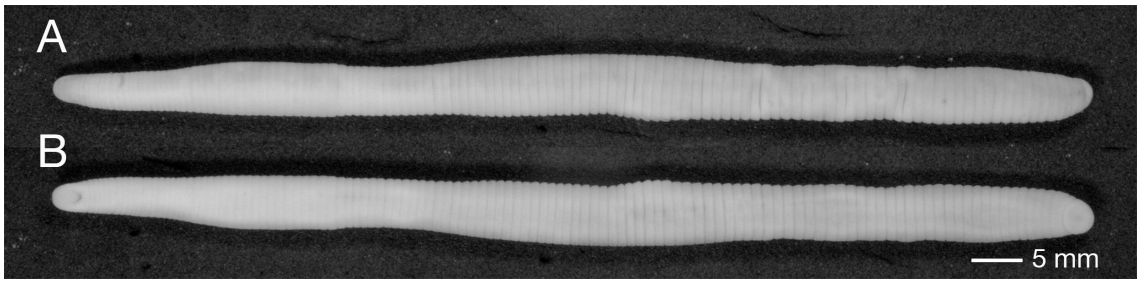
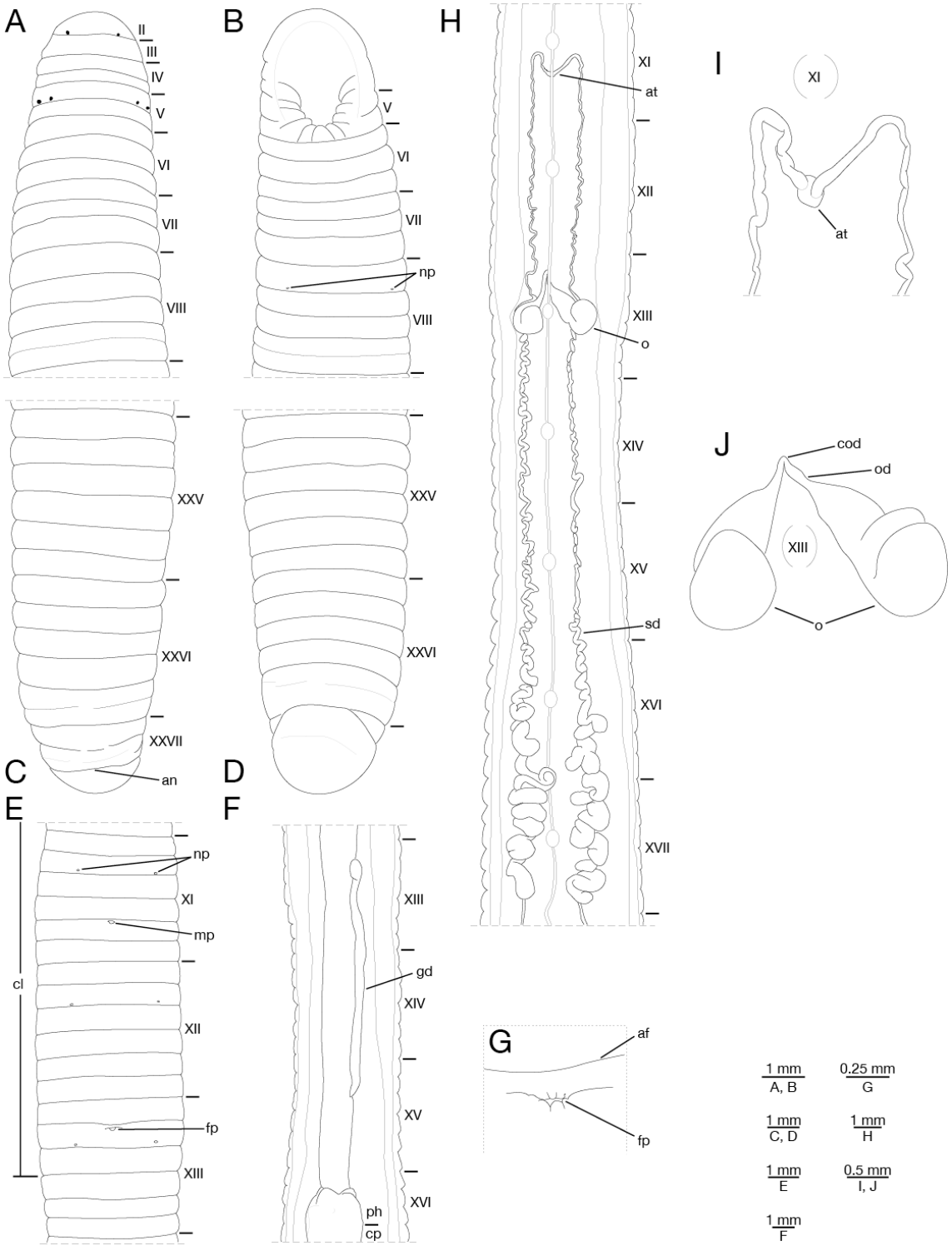


Fig. 26.


A
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XIII
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1 mm / H
1 mm / E
0.5 mm / I, J
1 mm / F

Fig. 27.

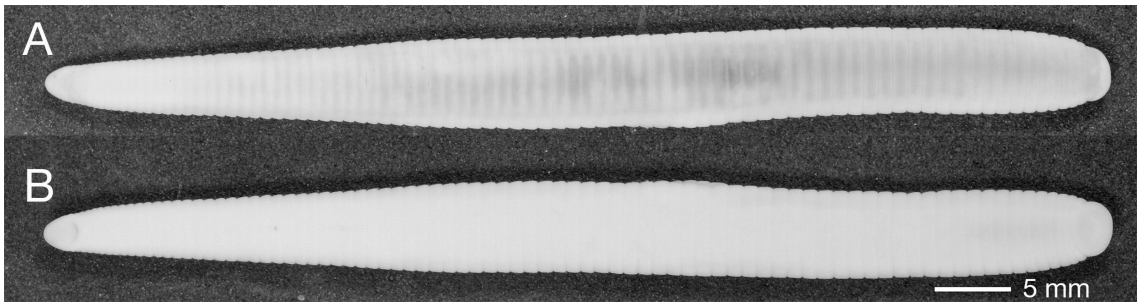
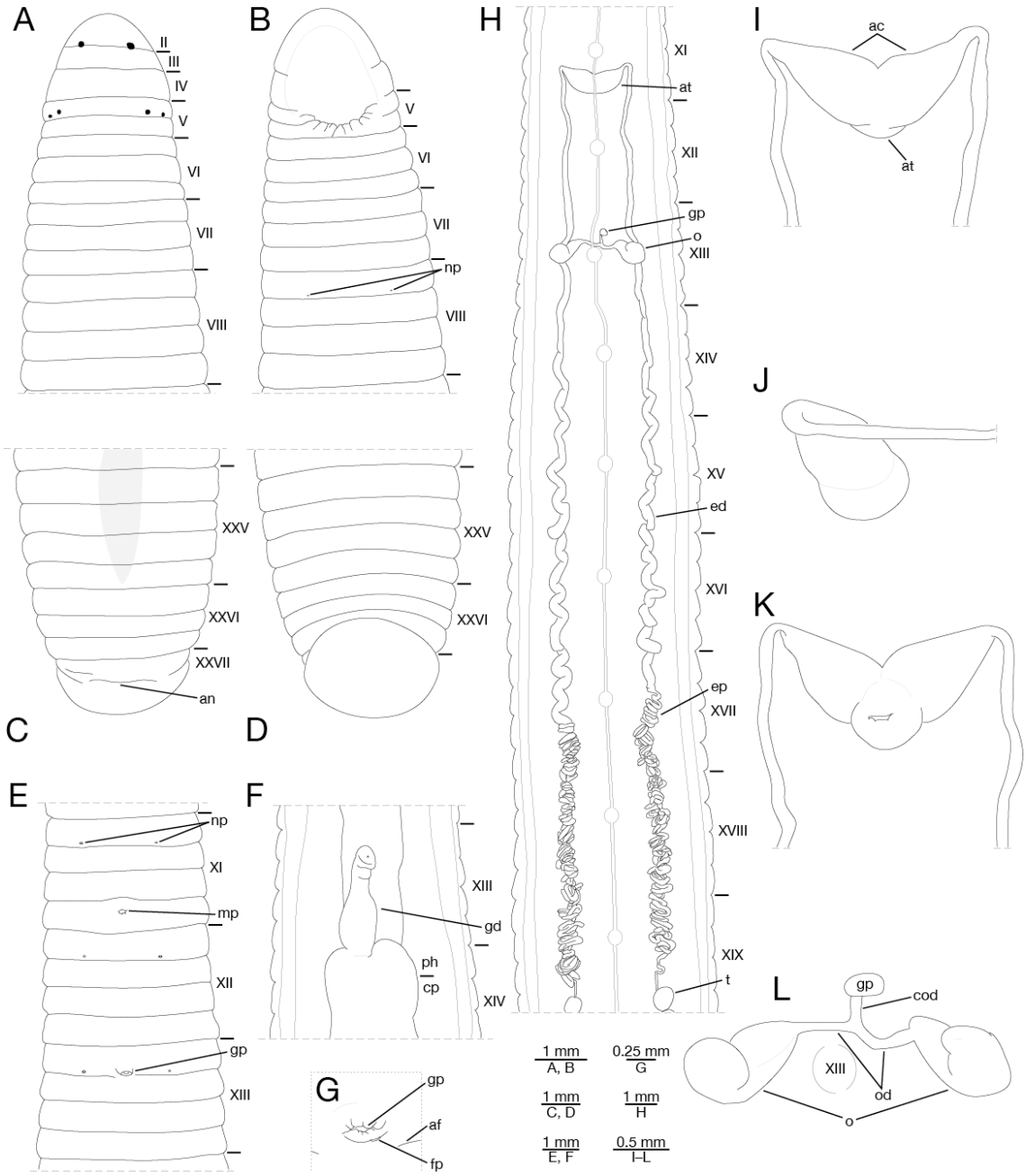


Fig. 28.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I-L

Fig. 29.

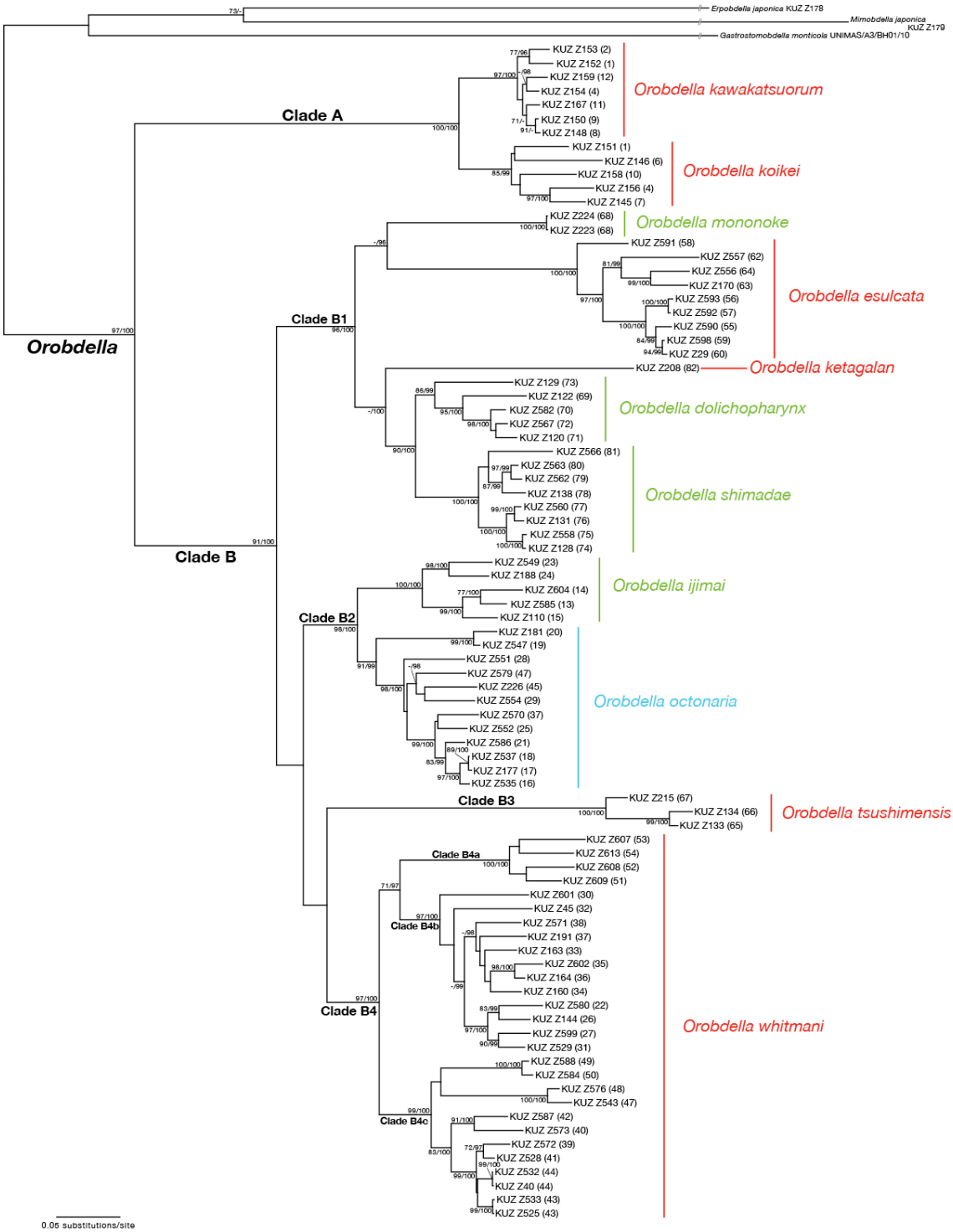
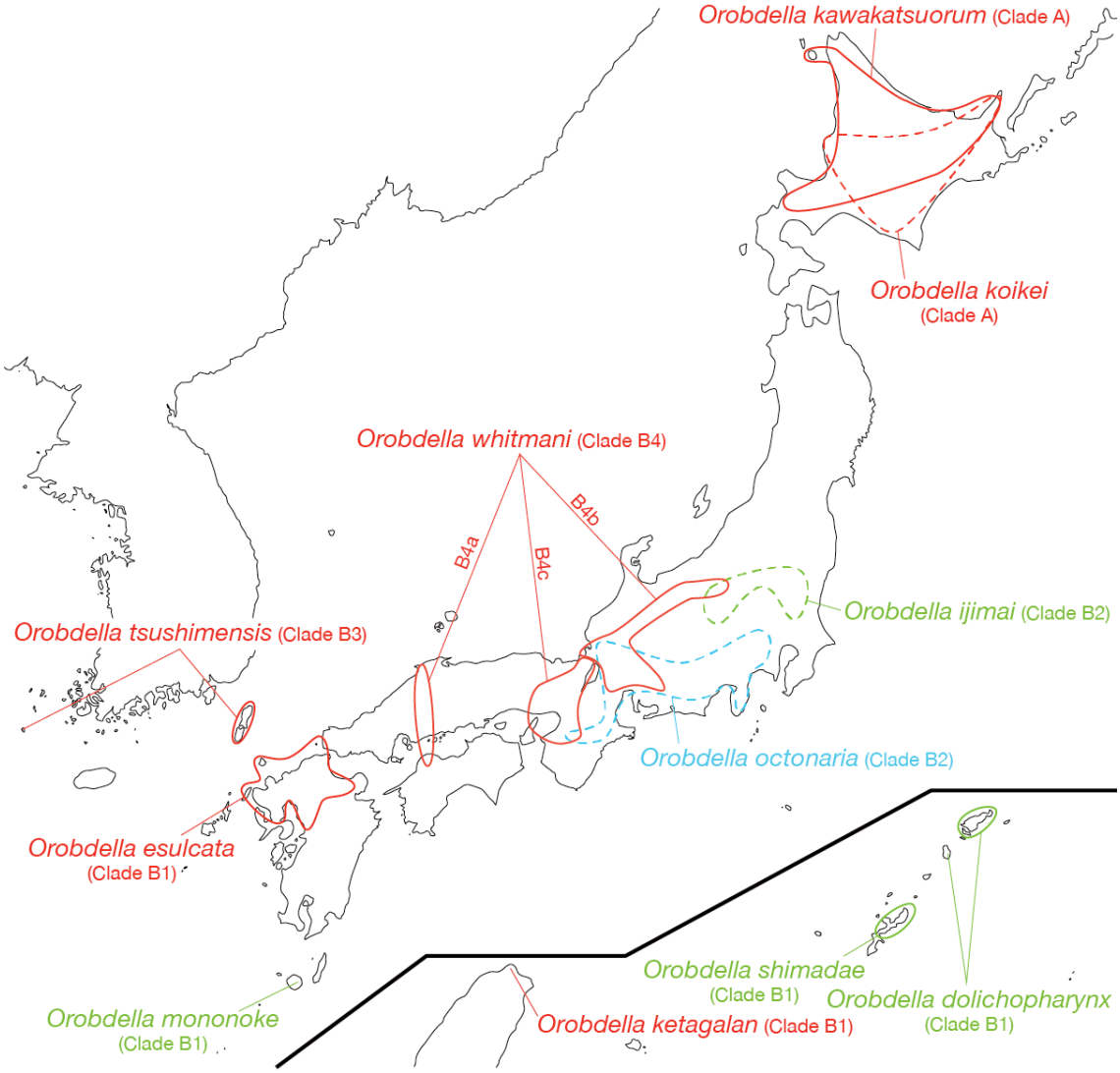

Erpobdella japonica KUZ Z178
Mimobdella japonica KUZ Z179
Gastrostomobdella monticola UNIMAS/A3/BH01/10
Clade A
KUZ Z153 (2)
KUZ Z152 (1)
KUZ Z159 (12)
KUZ Z154 (4)
KUZ Z167 (11)
KUZ Z150 (9)
KUZ Z148 (8)
Orobdella kawakatsuorum
KUZ Z151 (1)
KUZ Z146 (6)
KUZ Z158 (10)
KUZ Z156 (4)
KUZ Z145 (7)
Orobdella koikei
Orobdella
Clade B1
KUZ Z224 (68)
KUZ Z223 (68)
Orobdella mononoke
KUZ Z591 (58)
KUZ Z557 (62)
KUZ Z556 (64)
KUZ Z170 (63)
KUZ Z593 (56)
KUZ Z592 (57)
KUZ Z590 (55)
KUZ Z598 (59)
KUZ Z29 (60)
Orobdella esulcata
KUZ Z208 (82)
Orobdella ketagalan
KUZ Z129 (73)
KUZ Z122 (69)
KUZ Z582 (70)
KUZ Z567 (72)
KUZ Z120 (71)
Orobdella dolichopharynx
KUZ Z566 (81)
KUZ Z563 (80)
KUZ Z562 (79)
KUZ Z138 (78)
KUZ Z560 (77)
KUZ Z131 (76)
KUZ Z558 (75)
KUZ Z128 (74)
Orobdella shimadae
Clade B
KUZ Z549 (23)
KUZ Z188 (24)
KUZ Z604 (14)
KUZ Z585 (13)
KUZ Z110 (15)
Orobdella ijimai
Clade B2
KUZ Z181 (20)
KUZ Z547 (19)
KUZ Z551 (28)
KUZ Z579 (47)
KUZ Z226 (45)
KUZ Z554 (29)
KUZ Z570 (37)
KUZ Z552 (25)
KUZ Z586 (21)
KUZ Z537 (18)
KUZ Z177 (17)
KUZ Z535 (16)
Orobdella octonaria
Clade B3
KUZ Z215 (67)
KUZ Z134 (66)
KUZ Z133 (65)
Orobdella tsushimensis
Clade B4a
KUZ Z607 (53)
KUZ Z613 (54)
KUZ Z608 (52)
KUZ Z609 (51)
Clade B4b
KUZ Z601 (30)
KUZ Z45 (32)
KUZ Z571 (38)
KUZ Z191 (37)
KUZ Z163 (33)
KUZ Z602 (35)
KUZ Z164 (36)
KUZ Z160 (34)
Clade B4
KUZ Z580 (22)
KUZ Z144 (26)
KUZ Z599 (27)
KUZ Z529 (31)
KUZ Z588 (49)
KUZ Z584 (50)
KUZ Z576 (48)
KUZ Z543 (47)
Clade B4c
KUZ Z587 (42)
KUZ Z573 (40)
KUZ Z572 (39)
KUZ Z528 (41)
KUZ Z532 (44)
KUZ Z40 (44)
KUZ Z533 (43)
KUZ Z525 (43)
Orobdella whitmani
0.05 substitutions/site

Fig. 30.


Orobdella kawakatsuorum (Clade A)
Orobdella koikei
(Clade A)
Orobdella whitmani (Clade B4)
B4a
B4b
B4c
Orobdella ijimai (Clade B2)
Orobdella tsushimensis (Clade B3)
Orobdella octonaria (Clade B2)
Orobdella esulcata
(Clade B1)
Orobdella shimadae
(Clade B1)
Orobdella dolichopharynx
(Clade B1)
Orobdella mononoke
(Clade B1)
Orobdella ketagalan (Clade B1)

Appendix

Material examined

Orobdella whitmani Oka, 1895

Seventy-five specimens from Japan. Twenty specimens from Gifu Prefecture: fourteen specimens, UMUTZ-Ann-Hir-5-1, **lectotype**, KUZ Z18–Z20 (alt. 81 m, 35°25′40″N, 136°46′55″E), KUZ Z21 and Z22 (alt. 78 m, 35°25′40″N, 136°46′53″E), both dissected, KUZ Z23 (alt. 71 m, 35°25′40″N, 136°46′53″E), KUZ Z25 (alt. 35 m, 35°25′40″N, 136°46′54″E), dissected, KUZ Z26 (alt. 88 m, 35°25′39″N, 136°46′46″E), KUZ Z27 (alt. 30 m, 35°25′40″N, 136°46′54″E), dissected, KUZ Z43 (alt. 59 m, 35°25′38″N, 136°46′55″E), KUZ Z44 (alt. 59 m, 35°25′39″N, 136°46′54″E), KUZ Z45 (alt. 58 m, 35°25′39″N, 136°46′54″E), and KUZ Z46 (alt. 9 m, 35°25′58″N, 136°46′25″E), from Mt. Kinkazan, Gifu (locality no. 32); three specimens, KUZ Z161 and Z162 (alt. 280 m, 35°37′26″N, 136°39′36″E), and KUZ Z163 (alt. 266 m, 35°37′33″N, 136°39′20″E), dissected, from Neookudani, Sumoto (locality no. 33); KUZ Z160, dissected, from Nukumitoge, Sumoto (alt. 930 m, 35°46′31″N, 136°31′27″E; locality no. 34); KUZ Z529, dissected, from Kamitakaracho-sugoroku, Takayama (alt. 570 m, 36°18′56″N, 137°23′50″E; locality no. 31); KUZ Z602, dissected, from Kamiishizucho-tokiyama, Ogaki (alt. 300 m, 35°14′36″N, 136°23′55″E; locality no. 35); and KUZ Z603, from near the Matsuoike Pond, Gifu (alt. 108 m, 35°27′22″N, 136°48′17″E; locality no. 32). Twenty-one specimens from Kyoto Prefecture: twelve specimens, KUZ Z33, Z34 and Z37, and KUZ Z38 (alt. 103 m, 35°00′14″N, 135°47′08″E), dissected, KUZ Z35 and Z36 (alt. 104 m, 35°00′15″N, 135°47′07″E), KUZ Z39 (alt. 109 m, 35°00′14″N, 135°47′09″E), dissected, KUZ Z40 (alt. 108 m, 35°00′15″N, 135°47′08″E), dissected, KUZ Z41 (alt. 108 m, 35°00′14″N, 135°47′08″E), dissected, KUZ Z42 (alt. 106 m, 35°00′14″N, 135°47′07″E), KUZ Z47 (alt. 88 m, 35°00′15″N, 135°47′08″E), and KUZ Z50 (alt. 110 m, 35°00′13″N, 135°47′08″E), dissected, from Mt. Kodaijiyama, Kyoto (locality no. 44); three specimens, KUZ Z527 (alt. 435 m, 35°18′03″N, 135°44′06″E), KUZ Z587 (alt. 670 m, 35°20′43″N, 135°45′10″E), dissected, KUZ Z605 (35°20′53″N, 135°45′13″E), from Ashiu Forest Research Station, Nantan (locality no. 42); KUZ Z526 (alt. 431 m, 35°08′03″N, 135°45′51″E), KUZ Z533 (alt. 367 m, 35°08′08″N, 135°45′45″E), dissected, from Kibune, Kyoto (locality no. 43); KUZ Z525, dissected, from Hanasetoge, Kyoto (35.1°N, 135.5°E; locality no. 43); KUZ Z530, dissected, from Keihoku-kamiyugecho, Kyoto (alt. 600 m, 35°15′03″N, 135°42′42″E; locality no. 42);

KUZ Z532, dissected, from Mt. Nyakuojiyama, Kyoto (alt. 154 m, 35°00′51″N, 135°47′52″E; locality no. 44); KUZ Z568, from Kurama, Kyoto (35°07.4′N, 135°46.5′E; locality no. 43). Nine specimens from Shiga Prefecture: two specimens, KUZ Z164 (alt. 1018 m, 35°24′48″N, 136°25′11″E), dissected, KUZ Z165 (alt. 1368 m, 35°25′04″N, 136°24′18″E), from Mt. Ibukiyama, Maibara (locality no. 36); two specimens, KUZ Z191 (alt. 377 m, 35°39′38″N, 136°11′18″E), dissected, KUZ Z192 (alt. 366 m, 35°39′34″N, 136°11′32″E), from Yogocho-nakanokawachi, Nagahama (locality no. 37); KUZ Z528, dissected, from Mt. Shiratakiyama, Otsu (35°13′N, 135°53′E; locality no. 41); KUZ Z531, dissected, from Kutsukioisugi, Takashima (35°21′N, 135°46′E; locality no. 42); KUZ Z569, from Mt. Horaisan, Otsu (alt. 920 m, 35°13′21″N, 135°53′10″E; locality no. 41); KUZ Z572, dissected, from Choshigataki, Takashima (alt. 297 m, 35°30′07″N, 136°01′32″E; locality no. 39); and KUZ Z573, dissected, from Awagara, Takashima (alt. 410 m, 35°27′39″N, 135°58′09″E; locality no. 40). Seven specimens from Osaka Prefecture: three specimens, KUZ Z543 (alt. 659 m, 34°24′15″N, 135°39′58″E), dissected, KUZ Z544 (alt. 662 m, 34°24′16″N, 135°39′59″E), and KUZ Z578 (alt. 671 m, 34°24′14″N, 135°39′56″E), from Mt. Kongosan, Chihaya-akasaka (locality no. 47); three specimens, KUZ Z574 (alt. 350 m, 34°22′00″N, 135°26′01″E), KUZ Z575 (alt. 443 m, 34°21′43″N, 135°26′05″E), and KUZ Z576 (alt. 476 m, 34°21′40″N, 135°26′06″E), from Mt. Izumi-katsuragisan, Kishiwada (locality no. 48); and KUZ Z577, from Ishimigawa, Kawachinagano (alt. 491 m, 34°23′32″N, 135°38′46″E; locality no. 47). Four specimens from Hyogo Prefecture: two specimens, KUZ Z583, and KUZ Z584, dissected, from Mt. Jyoryujisan, Awaji, Awajishima Island (alt. 455 m, 34°30′35″N, 134°55′31″E; locality no. 50); and two specimens, KUZ Z588, dissected, and KUZ Z589, from Sumiyoshi Shrine, Sasayama (alt. 300 m, 35°01.4′N, 135°06.0′E; locality no. 49). Three specimens from Shimane Prefecture: KUZ Z606, from Mt. Hanatakasan, Izumo (alt. 102 m, 35°25′32″N, 132°44′59″E; locality no. 53); KUZ Z607, dissected, from Kawashimocho, Izumo (alt. 55 m, 35°26′00″N, 132°44′57″E; locality no. 53); and KUZ Z608, dissected, from Tainosuyama, Unnan (alt. 443 m, 35°07′29″N, 132°53′53″E; locality no. 52). Two specimens from along the Manzawa Forest Road, Nakanojyo, Gunma (alt. 1027 m, 36°41′13″N, 138°41′28″E; locality no. 22): KUZ Z580, dissected, and KUZ Z581. Two specimens from Kurobe, Toyama Prefecture (locality no. 27): KUZ Z599, dissected, from Babadani (alt. 740 m, 36°41′41″N, 137°40′29″E); and KUZ Z600, dissected, from

Kuronagi (36°47.1′N, 137°37.5′E). Two specimens from Mt. Takashiburoyama, Shobara, Hiroshima Prefecture (alt. 340 m, 34°46′03″N, 133°06′44″E; locality no. 51); KUZ Z609, dissected, and KUZ Z610. KUZ Z144, dissected, from Mt. Sunahachiyama, Nagano, Nagano Prefecture (alt. 1103 m, 36°41′40″N, 138°02′25″E; locality no. 26). KUZ Z571, dissected, from Mt. Nosakadake, Tsuruga, Fukui Prefecture (alt. 325 m, 35°36′15″N, 136°01′42″E; locality no. 38). KUZ Z601, dissected, from Mt. Rokusyosan, Toyota, Aichi Prefecture (alt. 438 m, 35°03′35″N, 137°17′01″E; locality no. 30). KUZ Z613, dissected, from Mt. Narabarasan, Imabari, Ehime Prefecture (alt. 843 m, 33°56′55″N, 132°56′24″E; locality no. 54).

Orobdella dolichopharynx Nakano, 2011

Thirteen specimens from three islands in the Ryukyu Islands, Kagoshima Prefecture, Japan. Eleven specimens from Amami-oshima Island: seven specimens, KUZ Z120, **holotype**, and KUZ121 (alt. 448 m, 28°17′11″N, 129°18′56″E), both dissected, KUZ Z115 (alt. 520 m, 28°17′27″N, 129°18′58″E), KUZ Z116 (alt. 536 m, 28°17′29″N, 129°19′00″E), dissected, KUZ Z117 (alt. 440 m, 28°17′06″N, 129°18′55″E), KUZ Z118 (alt. 606 m, 28°17′42″N, 129°19′06″E), dissected, and KUZ Z119 (alt. 434 m, 28°17′08″N, 129°18′55″E), dissected, from Mt. Yuwandake, Uken (locality no. 71); three specimens, KUZ Z122, dissected, KUZ Z123, and KUZ Z124, from Kinsakubaru, Amami (locality no. 69); and KUZ Z582, dissected, from Yakugachigawa, Setouchi (28°13′34″N, 129°21′19″; locality no. 70). KUZ Z129, dissected, from Mt. Inokawadake, Tokunoshima, Tokunoshima Island (alt. 204 m, 27°45′54″N, 128°59′14″E; locality no. 73). KUZ Z567, dissected, from Seso, Setouchi, Kakeromajima Island (28°07′09″N, 129°14′25″E; locality no. 72).

Orobdella esulcata Nakano, 2010

Twenty-three specimens from Kyushu, and its peripheral islands, Japan. Eight specimens from Mt. Kimposan, Kumamoto, Kumamoto Prefecture (locality no. 60): KUZ Z29 (alt. 412 m, 32°48′36″N, 130°38′29″E), **holotype**, dissected, KUZ Z28 (alt. 373 m, 32°48′34″N, 130°38′39″E), KUZ Z30 (alt. 481 m, 32°48′42″N, 130°38′27″E), dissected, KUZ Z31 and Z32 (alt. 479 m, 32°48′41″N, 130°38′27″E), dissected, KUZ Z48 (alt. 358 m, 32°48′34″N, 130°38′39″E), KUZ Z49 (alt. 355 m, 32°48′52″N, 130°38′43″E), and KUZ Z51 (alt. 504 m, 32°48′43″N, 130°38′28″E). Six specimens

from Nagasaki Prefecture: four specimens, KUZ Z170 (alt. 192 m, 33°44′28″N, 129°42′15″E), dissected, KUZ Z171 (alt. 209 m, 33°44′29″N, 129°42′19″E), dissected, KUZ Z172 (alt. 143 m, 33°44′28″N, 129°42′14″E), and KUZ Z176 (alt. 142 m, 33°44′28″N, 129°42′14″E), from Mt. Takenotsuji, Iki, Ikinoshima Island (locality no. 63); KUZ Z556, dissected, from Mt. Yasumandake, Hirado, Hiradojima Island (alt. 500 m, 33°20.1′N, 129°28.1′E; locality no. 64); and KUZ Z557, dissected, from Mt. Taradake, Omura (32°58.2′N, 130°05.2′E; locality no. 62). Six specimens from Fukuoka Prefecture: four specimens, KUZ Z594 (alt. 397 m, 33°28′52″N, 130°17′54″E), KUZ Z595 (alt. 399 m, 33°28′52″N, 130°17′55″E), KUZ Z596 (alt. 401 m, 33°28′52″N, 130°17′53″E), dissected, and KUZ Z597 (alt. 409 m, 33°28′50″N, 130°17′53″E), from Mt. Kanayama, Itoshima (locality no. 61); KUZ Z591, dissected, from Mt. Fukuchiyama, Kitakyushu (alt. 193 m, 33°44′27″N, 130°50′17″E; locality no. 58); and KUZ Z598, dissected, from Ukihamachi-tagomori, Ukiha (alt. 403 m, 33°14′45″N, 130°51′20″E; locality no. 59). Two specimens from Oita Prefecture: KUZ Z592, dissected, from Mt. Nakamadonno-hatayama, Nakatsu (alt. 676 m, 33°27′23″N, 131°01′45″E; locality no. 57); and KUZ Z593, dissected, from Aso, Usa (alt. 121 m, 33°28′05″N, 131°16′03″E; locality no. 56). KUZ Z590, dissected, from Futaoijima Island, Shimonoseki, Yamaguchi Prefecture (alt. 92 m, 34°06′14″N, 130°47′29″E; locality no. 55).

Orobdella kawakatsuorum Richardson, 1975

Twenty-three specimens from Hokkaido, and its peripheral island, Japan. Seven specimens from Sapporo (locality no. 11): NSMT-An 53, **holotype**, dissected, from the home garden of Professor Masaharu Kawakatsu; six specimens, KUZ Z24, dissected, and KUZ Z140 (alt. 50 m, 43°03′07″N, 141°18′32″E), KUZ Z166, and KUZ Z167 (alt. 34 m, 43°03′09″N, 141°18′43″E), dissected, KUZ Z168, and KUZ Z169 (alt. 29 m, 43°03′09″N, 141°18′37″E), dissected, from Maruyama Park. Six specimens from Sounkyo, Kamikawa (locality no. 4): KUZ Z154, dissected, and KUZ Z155 (alt. 705 m, 43°43′33″N, 142°57′32″E), KUZ Z183 (alt. 674 m, 43°43′27″N, 142°56′52″E), KUZ Z184 and Z185 (alt. 678 m, 43°43′23″N, 142°56′53″E), and KUZ Z187 (alt. 758 m, 43°43′17″N, 142°56′52″E). Two specimens from Mt. Asahidake, Higashikawa (locality no. 5): KUZ Z141 (alt. 1090 m, 43°38′49″N, 142°47′44″E), dissected, and KUZ Z142 (alt. 1120 m, 43°39′07″N, 142°48′06″E). Two specimens from near Kabutonuma Park,

Toyotomi (alt. 16 m, 45°13′13″N, 141°41′04″E; locality no. 8): KUZ Z147, and KUZ Z148, dissected. Two specimens from Mt. Rishirizan, Rishirifuji, Rishirito Island (alt. 914 m, 45°12′00″N, 141°14′16″E; locality no. 9): KUZ Z149, and KUZ Z150, dissected. KUZ Z143, dissected, from Nukabira, Kamishihoro (alt. 490 m, 43°22′03″N, 143°11′37″E; locality no. 3). KUZ Z152, dissected, from Mt. Rausudake, Shari (alt. 630 m, 44°06′05″N, 145°06′05″; locality no. 1). KUZ Z153, dissected, from Mt. Meakandake, Ashoro (alt. 755 m, 43°23′42″N, 143°59′14″E; locality no. 2). KUZ Z159, dissected, from near the Shinsennuma Pond, Kyowa (alt. 781 m, 42°46′10″N, 140°35′34″E; locality no. 12).

Orobdella ketagalan Nakano & Lai, 2012

Six specimens from Taipei City, Taiwan. Five specimens collected from Yangmingshan National Park (locality no. 82): KUZ Z208, **holotype**, and KUZ Z209 (alt. 779 m, 25°11′07″N, 121°31′10″E), both dissected, KUZ Z207 (alt. 776 m, 25°09′49″N, 121°33′10″E), KUZ Z210 (alt. 600 m, 25°11′11″N, 121°31′10″E), dissected, and KUZ Z211 (alt. 737 m, 25°10′55″N, 121°30′50″E). KUZ Z179, from Jinsan Township (alt. 739 m, 25°11′01″N, 121°30′54″E; locality no. 82).

Orobdella koikei Nakano, 2012

Seven specimens from Hokkaido, Japan. Three specimens collected from Sounkyo, Kamikawa (43°43′22″N, 142°56′51″E; locality no. 4): KUZ Z156, **holotype**, dissected, and KUZ Z157 (alt. 721 m), and KUZ Z186 (alt. 717 m). KUZ Z145, dissected, form Hiratori (alt. 220 m, 42°40′49″N, 142°25′26″E; locality no. 7). KUZ Z146, dissected, from Mt. Pisenaiyama, Hidaka (42°24′N, 142°38′E; locality no. 6). KUZ Z151, dissected, from Mt. Rausudake, Shari (alt. 422 m, 44°06′22″N, 145°05′36″E; locality no. 1). KUZ Z158, dissected, from Mt. Shokanbetsudake, Mashike (alt. 288 m, 43°46.1′N, 141°30.4′E; locality no. 10).

Orobdella ijimai Oka, 1895

Thirteen specimens collected from Honshu, Japan. Six specimens from Nikko, Tochigi Prefecture (locality no. 15): KUZ Z108 (alt. 960 m, 36°47′01″N, 139°34′54″E), dissected, KUZ Z109 (alt. 946 m, 36°46′59″N, 139°34′55″E), KUZ Z110, dissected, and KUZ Z111 (alt. 940 m, 36°46′59″N, 139°34′56″E), KUZ Z112 (alt. 990 m,

36°47′08″N, 139°34′54″E), and KUZ Z113 (alt. 1014 m, 36°47′13″N, 139°34′51″E), dissected. Three specimens from Mt. Arafuneyama, Saku, Nagano Prefecture (locality no. 24): KUZ Z188 (alt. 1129 m, 36°12′26″N, 138°37′45″E), dissected, KUZ Z189 (alt. 1136 m, 36°12′27″N, 138°37′45″E), and KUZ Z190 (alt. 1140 m, 36°12′26″N, 138°37′46″E). Two specimens from Shibutoge, Nakanojyo, Gunma Prefecture (alt. 2139 m, 36°39′58″N, 138°32′11″E; locality no. 23): KUZ Z549, dissected, and KUZ Z550. Two specimens from Ibaraki Prefecture: KUZ Z585, dissected, from Mt. Yamizosan, Daigo (36°55.5′N, 140°16.2″E; locality no. 13); KUZ Z604, dissected, from Mt. Wagakunisan, Kasama (36°19′N, 140°12′E; locality no. 14).

Orobdella mononoke Nakano, 2012

Five specimens from Yakushima, Yakushima Island in the Ryukyu Islands, Kagoshima Prefecture, Japan (locality no. 68). Three specimens from Shiratani-unsuikyo: KUZ Z224 (alt. 648 m, 30°22′47″N, 130°34′29″E), **holotype**, KUZ Z221 (alt. 649 m, 30°22′52″N, 130°34′41″E), both dissected, and KUZ Z225 (alt. 646 m, 30°22′45″N, 130°34′29″E). Two specimens from Kusugawa (alt. 363 m): KUZ Z222 (30°23′46″N, 130°35′15″E), and KUZ Z223 (30°23′45″N, 130°35′15″E), dissected.

Orobdella octonaria Oka, 1895

Twenty-five specimens from Honshu, Japan. Three specimens from Mt. Yusakayama, Hakone, Kanagawa Prefecture (locality no. 20): NSMT-An 415, **lectotype**, dissected, KUZ Z181 (alt. 585 m, 35°14′03″N, 139°04′12″E), dissected, and KUZ Z182 (alt. 577 m, 35°14′04″N, 139°04′13″E). Ten specimens from Uenohara, Yamanashi Prefecture: eight specimens, KUZ Z537, dissected, and KUZ Z538 (alt. 533 m, 35°40′38″N, 139°03′15″E), KUZ Z539 and Z540 (alt. 508 m, 35°41′14″N, 139°02′07″E), dissected, KUZ Z541 (alt. 480m, 35°41′18″N, 139°02′47″E), KUZ Z542 (alt. 422 m, 35°41′02″N, 139°03′27″E), KUZ Z546 and Z548 (alt. 830 m, 35°39′33″N, 139°02′10″E), from Nishihara (locality no. 18); two specimens, KUZ Z545, and KUZ Z547, dissected, from Ono (alt. 300 m, 35°37′06″N, 139°03′29″E; locality no. 19). Four specimens from Tokyo: three specimens, KUZ Z177 (alt. 253 m, 35°42′56″N 139°12′13″), dissected, KUZ Z534 (alt. 231 m, 35°43′19″N, 139°11′59″E), and KUZ Z536 (alt. 186 m, 35°43′18″N, 139°12′15″E), from Akiruno (locality no. 17); KUZ Z535, dissected, from Oume (alt. 344 m, 35°48′18″N, 139°13′48″E; locality no. 16). Three specimens from

Shizuoka Prefecture: two specimens, KUZ Z552, dissected, and KUZ Z 553, from Mochimune-shiroyamacho, Shizuoka (34°55′N, 138°21′E; locality no. 25); KUZ Z586, dissected from Namesawa Gorge, Izu (alt. 558 m, 34°50′35″N, 138°54′40″E; locality no. 21). KUZ Z551, dissected, from Mt. Enasan, Nakatsugawa, Gifu Prefecture (alt. 1400 m, 35°25′11″N, 137°36′37″E; locality no. 28). KUZ Z554, dissected, from Mt. Horaijisan, Shinshiro, Aichi Prefecture (34°58′N, 137°35′E; locality no. 29). KUZ Z226, dissected, from Fujigano, Taiki, Mie Prefecture (alt. 200 m, 34°19′28″N, 136°24′12″E; locality no. 45). KUZ Z570, dissected, from Yogocho-nakanokawachi, Nagahama, Shiga Prefecture (alt. 361 m, 35°39′43″N, 136°11′36″E; locality no. 37). KUZ Z579, dissected, from Mt. Kongosan, Chihaya-akasaka, Osaka Prefecture (alt. 671 m, 34°24′14″N, 135°39′56″E; locality no. 47).

Orobdella shimadae Nakano, 2011

Twenty specimens collected from Okinawajima Island in the Ryukyu Islands, Okinawa Prefecture, Japan. KUZ Z128, **holotype**, dissected, from Okuyona, Kunigami (alt. 100 m, 26°49.0′N, 128°16.5′E; locality no. 74). Six specimens from Benoki, Kunigami (locality no. 76): KUZ Z125, KUZ Z130, KUZ Z131 (alt. 176 m, 26°47′20″N, 128°15′22″E), dissected, KUZ Z132 (alt. 178 m, 26°47′20″N, 128°15′21″E), KUZ Z139 (26°47.0′N, 128°16.3′E), and KUZ Z555 (26°46′N, 128.2°E). Four specimens from Higashi (locality no. 78): KUZ Z126, KUZ Z127, dissected, KUZ Z137, and KUZ Z138 (26°40.2′N, 128°11.2′E), dissected. Three specimens from Mt. Katsuudake, Motobu (locality no. 80): KUZ Z563 (alt. 301 m, 26°37′53″N, 127°56′16″E), dissected, KUZ Z564 and Z565 (alt. 332 m, 26°37′55″N, 127°56′13″E). Two specimens from along the Chinufuku Forest Road, Mt. Nishimedake, Kunigami (locality no. 75): KUZ Z558 (alt. 327 m, 26°48′27″N, 128°16′06″E), dissected, and KUZ Z559 (alt. 314 m, 26°48′26″N, 128°16′08″E). Two specimens from Mt. Yonahadake, Kunigami (alt. 281 m, 26°43′44″N, 128°12′44″E; locality no. 77): KUZ Z560, dissected, and KUZ Z561. KUZ Z562, dissected, from near the Haneji Dam, Nago (alt. 93 m, 26°35′22″N, 128°01′44″E; locality no. 79). KUZ Z804, dissected, from Mt. Ishikawadake, Onna (alt. 70 m, 26°27′07″N, 127°49′53″E; locality no. 81).

Orobdella tsushimensis Nakano, 2011

Ten specimens. Seven specimens collected from Tsushima Island, Nagasaki Prefecture,

Japan: three specimens, KUZ Z134, **holotype**, and KUZ Z135 (alt. 69 m, 34°15′17″N, 129°17′17″E), both dissected, and KUZ Z136 (alt. 72 m, 34°15′17″N, 129°17′14″E), dissected, from Mitsushimacho-Kechi, Tsushima (locality no. 66); four specimens, KUZ Z133 (alt. 133 m, 34°34′40″N, 129°22′29″E), dissected, KUZ Z173 (alt. 128 m, 34°34′37″N, 129°22′25″E), KUZ Z174 and Z175 (34°34′N, 129°22′E), from Mt. Mitake, Tsushima (locality no. 65). Three specimens from Mt. Doiksilsan, Gageodo Island, Heuksan-myeon, Sinan-gun, Jeollanam-do, the Republic of Korea (alt. 639 m, 34°04′35″N, 125°06′27″E; locality no. 67): KUZ Z213, dissected, KUZ Z214, and KUZ Z215, dissected.